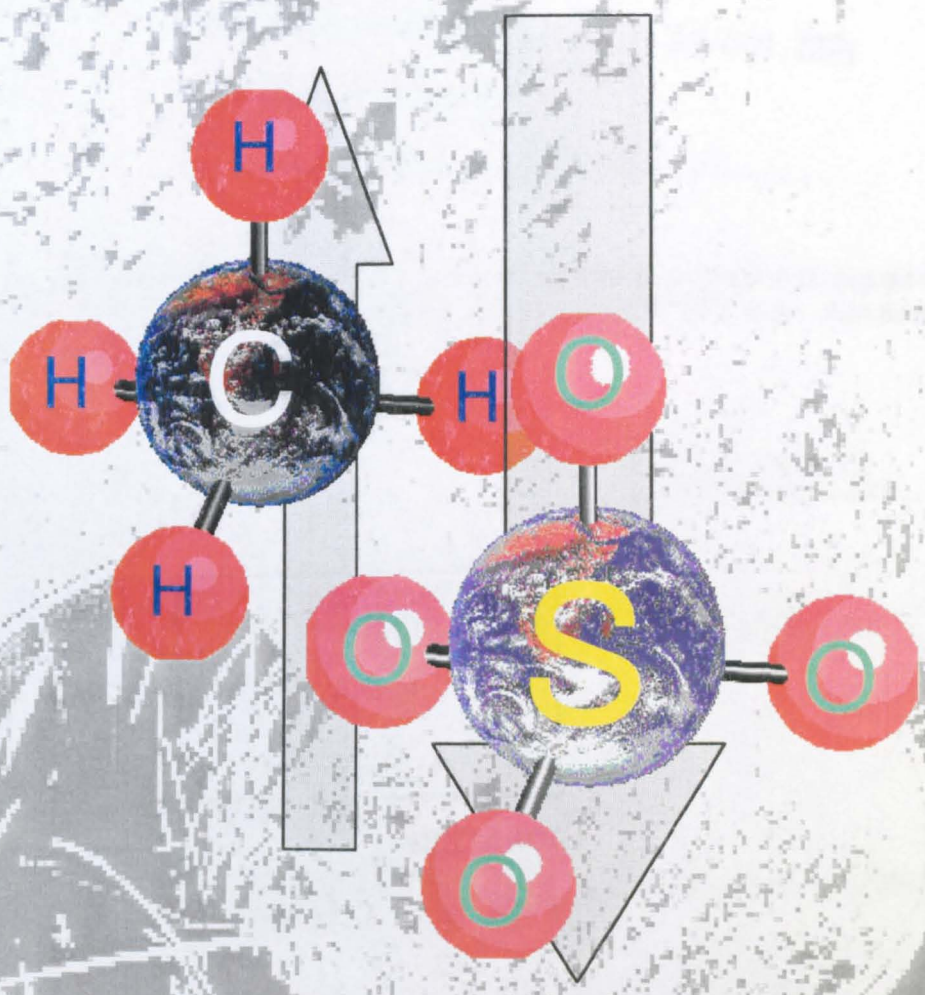
Acid rain links to CH_4 emissions from wetlands

Thesis



acid rain links to CH_4 emissions from wetlands

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Abstract

A variety of approaches, spanning a range of spatial and temporal scales, were applied to the investigation of the effects of low dose SO_4^{2-} deposition, at rates comparable to those experienced in acid rain impacted areas, on methane (CH_4) emissions from natural wetlands.

Over two years of experimental manipulation of SO_4^{2-} deposition to a peatland in northeast Scotland, CH_4 emissions were suppressed by around 40%. There was no significant difference in suppression of CH_4 flux within the SO_4^{2-} deposition range of 25-100 kg-S $ha^{-1}yr^{-1}$. In a similar short-term controlled environment SO_4^{2-} manipulation experiment, the suppressive effect of SO_4^{2-} was found to be independent of the simulated SO_4^{2-} deposition rate within a range of 15-100 kg-S $ha^{-1}yr^{-1}$. The possibility that suppression of CH_4 fluxes may have been the result of a 'salt effect' was ruled out. Both temperature and water table controlled the extent of CH_4 flux suppression in acid rain impacted wetlands.

Sulfate reduction potential in SO_4^{2-} treatments were found to be 10 times larger than in control plots, suggesting that long-term suppression of CH_4 fluxes is the result of the formation of an enlarged population of competitively superior sulfate reducing bacteria.

SO_4^{2-} concentrations were smaller in peat pore water from SO_4^{2-} treatments than from controls. This is possibly the result of a stimulated SO_4^{2-} reducing community scavenging available SO_4^{2-}, thereby decreasing concentrations to below ambient levels.

In northern peatlands (>50°) the effect of SO_4^{2-} deposition at 1990 rates may have been sufficient to reduce emissions from these systems by around 15% annually. Globally, the effect of acid rain SO_4^{2-} deposition may be sufficient to reduce CH_4 emissions by as much as 22-28 Tg by 2030, which places this interaction within the same size category as many other components of the global CH_4 budget that have received far greater attention.

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This thesis is dedicated to the memory of my father,

Gaëtan Gauci

1941-2000

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List of Abbreviations

AVS	Acid-volatile sulfur
CEH	Centre for Ecology and Hydrology
ECMWF	European Centre for Medium-Range Weather Forecasting
FID	Flame Ionisation Detector
GCM	General Circulation Model
GHG	Greenhouse gas
MLURI	Macaulay Land Use Research Institute
MOP	Methane Oxidation Potential
MPB	Methane Producing Bacteria
MPP	Methane Production Potential
NPP	Net Primary Productivity
NASA GISS	National Aeronautics and Space Administration, Goddard Institute for Space Studies
$PVCH_4$	Percent Variation in CH_4
S^o	Elemental S
SRB	Sulfate Reducing Bacteria
SRP	Sulfate Reducing Potential
TIS	Total Inorganic Sulfur
TOS	Total Organic Sulfur

Chapter One

Introduction

1.1 General Overview.

As the most abundant hydrocarbon in the atmosphere and a powerful greenhouse gas, methane (CH_4) plays an important role in both the chemical composition and radiative balance of the earth's atmosphere. It is necessary to improve understanding of sources and sinks as well as processes governing the interaction of methane with other biogeochemical cycles if we are to better understand the function of the earth as a system and the role human activity may play in affecting the earth's natural balance.

CH_4 is produced by a number of natural and anthropogenic sources. Natural freshwater wetlands are the largest individual source (IPCC, 1995) where the gas is formed in the terminal stage of anaerobic decomposition by a group of bacteria known as methanogens (or methane producing bacteria – MPB). Anthropogenic methane sources, however, provide the majority of methane to the atmosphere, which has lead to a dramatic increase in the concentration of atmospheric methane since the onset of the industrial era

(Rasmussen and Khalil 1984; Blunier *et al.*, 1993; Etheridge *et al.*, 1998). Although this has been the overall trend during the past century there has recently been a slow down in the rate at which atmospheric methane is accumulating (fig 1.2, Dlugokencky *et al* 2001). The reasons for this are as yet unknown.

In coastal wetlands and wetlands overlying sulfate-rich deposits, methane emissions are very much smaller than otherwise comparable freshwater wetlands (Bartlett *et al., 1987;* Rejmankova and Post, 1996; Reeve *et al.,* 1996). In sulfate-rich environments this is thought to be due to microbial competition favouring sulfate reduction as an energetically more efficient means of carbon degradation than methanogenesis (Abram and Nedwell, 1978; Schonheit *et al.,* 1982; Kristjansson *et al.,* 1982.). There is also evidence that methane may be consumed anaerobically by a consortia of micro-organisms which may include sulfate-reducing bacteria (SRB) as well as MPB (Hoehler *et al.,* 1994; Hinrichs. *et al.* 1999; Boetius *et al.,* 2000).

Research has shown that in freshwaters, competition, favouring SRB over MPB may also occur when sulfate supply has been increased (Lovley and Klug 1983; Scholten and Stams 1995, Raskin *et al.,* 1996). Natural freshwater wetlands and peatlands may receive sulfate from rainwater at very low concentrations (Klinger and Erickson, 1997 and references therein). Increases in the anthropogenic output of sulfur over the past century, through fossil fuel combustion, however, is likely to have perturbed this natural input of sulfur to these systems particularly in those areas most affected by 20^{th} century industrialisation. These regions include North America and Western Europe and, to a growing extent Asia (Rodhe, 1999). Indeed, as pollution legislation limits the input of sulfur species to the atmosphere in western industrialised regions, aggressive economic growth in Asia may result in increased deposition of sulfur species to areas previously unaffected by pollution

(Bhatti *et al.*, 1992). There is therefore potential for pollutant S deposition to perturb the methane cycle by reducing the output of the largest methane source. This mechanism has only received limited detailed attention with either laboratory-based experiments (Nedwell and Watson, 1995; Fowler *et al.*, 1995; Watson and Nedwell, 1998) or short-term field experiments (Dise and Verry, in press). The purpose of this investigation is, therefore, to improve understanding of this potentially important uncertainty in the global methane cycle.

1.2 Atmospheric CH_4 and the greenhouse effect.

1.2.1 Climate Forcing

Concentrations of atmospheric methane (CH_4) have grown from pre-industrial concentrations of around 700 ppbv (IPCC 1995) to 1760 ppbv in 1999 (Dlugokencky *et al.*, in review). The gas strongly absorbs infrared radiation at a wavelength of around 7.7 μm making it 21 times more powerful a greenhouse gas than CO_2 on a molecule for molecule basis (Lelieveld *et al.*, 1998). Indirectly, CH_4 can lead to the production of tropospheric ozone (O_3) (when oxidised in the presence of NO_x compounds) and stratospheric water vapour, which are also greenhouse gases. Lelieveld *et al* (1998) estimate the total direct and indirect climate forcing of CH_4 to be 0.57 Wm^{-2}, or 35% that of CO_2 between the years 1850 and 2000. More recently, Hansen *et al* (2000) estimated a far larger impact of CH_4 with an estimated direct and indirect climate forcing totalling 0.7 Wm^{-2}, or 50% that of CO_2 during the same period (fig 1.1).

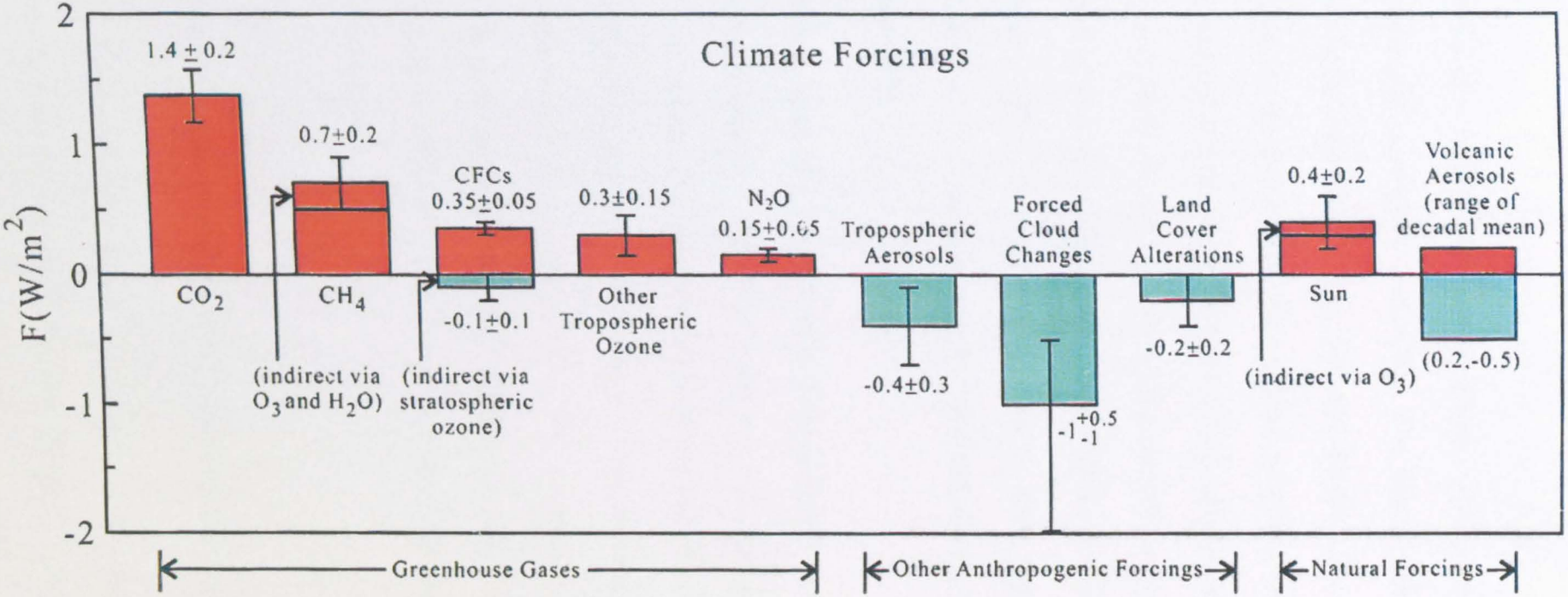

Figure 1.1: Estimated climate forcings between 1850 and 2000. Bar heights represent a mid-range estimate and error bars show the possible range of values. From Hansen *et al.* (2000)

1.2.2 Atmospheric chemistry and lifetime of CH_4

As a chemically reactive atmospheric species, CH_4 plays an important part in many tropospheric and stratospheric reactions. The largest sink for atmospheric CH_4 is its oxidation in the atmosphere by the hydroxyl radical (OH), which is highly reactive and responsible for the degradation of a number of greenhouse gases (IPCC 1995). The species produced through the destruction of CH_4 via oxidation by OH include CO, CO_2, H_2O, H_2 and CH_2O although ultimately, complete oxidation of CH_4 results in CO_2 and H_2O (Cicerone and Oremland 1988). O_3 is also produced when CH_4 is oxidised in the presence of sufficient NO_x; otherwise, if insufficient NO_x concentrations exist (threshold concentration of 10-15 ppt) O_3 may be consumed during CH_4 oxidation (Cicerone and Oremland 1988).

The different oxidation pathways cumulatively influence the lifetime, or 'turnover time' of CH_4 in the atmosphere. Since OH is the main CH_4 removal pathway, its consumption through the oxidation of CH_4 limits the atmospheric lifetime of OH to a few seconds. This feeds back on CH_4 concentrations by reducing the potential for further oxidation of CH_4, thereby extending the CH_4 lifetime (IPCC 1995). Lelieveld *et al.* (1998) suggest that over the last 150 years the chemical lifetime of CH_4 (currently 7.9 years) has been extended by as much as 25 to 30% due to the effect of increased atmospheric CH_4 on the depletion of OH concentrations.

1.3 Recent trends in the concentration of atmospheric CH_4

The use of ice cores as archives of past atmospheric constituents has enabled us to understand both natural changes in the atmospheric CH_4 burden associated with glacial /

interglacial cycles and other periods of climate change (e.g. the Little Ice Age) as well as the broad impact of anthropogenic activity on the atmospheric CH_4 balance (Chappellaz *et al.*, 1990; Chappellaz *et al.*, 1993; Blunier *et al.*, 1993; Etheridge *et al.*, 1998). In more recent times global monitoring networks have provided a high-resolution record of changes in CH_4 concentration since the early 1980's (Dlugokencky *et al.*, 1994). These have shown that, although atmospheric CH_4 concentrations continue to increase, the rate at which concentrations increase has declined over the last 20 years (Dlugokencky *et al.*, 1998). Barring two anomalous CH_4 growth periods, the slowdown continues to this day (fig. 1.2, Dlugokencky *et al., in press*). The reasons behind this slowdown are not understood although they will involve either reductions in the growth of CH_4 sources or increases in sinks.

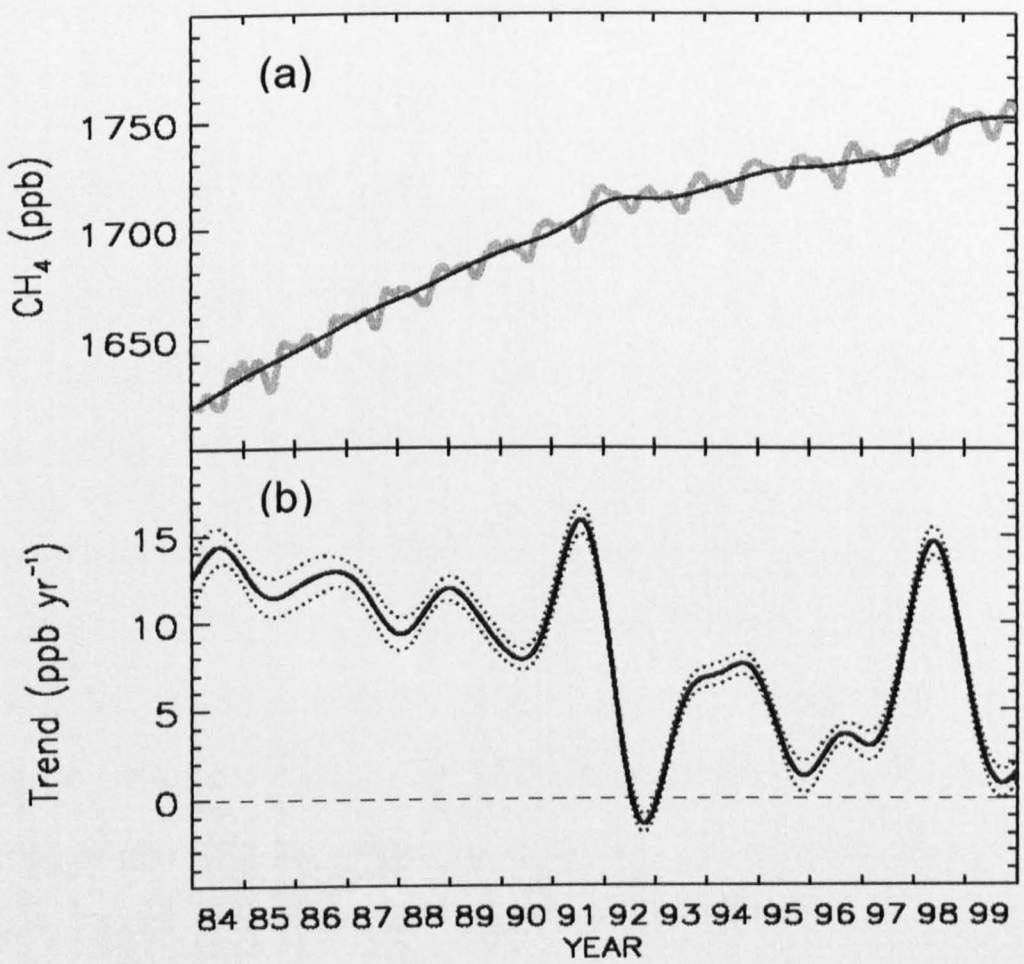

Figure 1.2: (a) Globally averaged CH_4 mole fractions. Solid line is the deseasonalized trend. (b) Globally averaged instantaneous growth rate determined as the time-derivative of the trend line in (a)(solid line) and its uncertainties (dotted line). [*Dlugokencky et al., 2001*]

It has been hypothesised that with the atmospheric OH sink remaining constant (Prinn *et al.*, 1995) the reduction in CH_4 growth rate may be due to stabilisation of the global CH_4 source. The slowdown may thus reflect approach to a steady state over a period of time, which reflects the atmospheric lifetime of CH_4 (Dlugokencky *et al.*, 1998). Isotopic analysis of CH_4 in archived air supports this hypothesis (Francey *et al.*, 1999). A problem with this hypothesis, however, is that CH_4 emissions from wetlands, the largest source, are thought to have increased over this time in response to climate warming during this time (Russell *et al.*, 2000; Walter unpublished data, see chapter 6 for further detail). If the isotopic data and interpretation are correct, either other large sources are decreasing at a rate commensurate to that of the expected increase in wetland emissions (and there is no evidence to suggest that they have) or wetland CH_4 emissions are not increasing. This may be due to some additional factor suppressing CH_4 emissions.

1.4 The global CH_4 budget

The sources and sinks of atmospheric CH_4 are summarized in table 1 (IPCC, 1995). The size of the wetland CH_4 source is such (table 1) that is will be discussed in detail in section 1.5. Here I provide a brief overview of the other components of the CH_4 budget.

Some components of the CH_4 budget have been better constrained than others. CH_4 from rice paddies, for example was previously estimated to contribute around 60Tg CH_4 (IPCC 1995). Recent work analysing the extensive measurements of rice CH_4 emission suggests that the rice source strength is far smaller (~30Tg, Dernier van der Gon pers. comm.). In these systems CH_4 is produced by the anaerobic degradation of organic matter in flooded soils. Factors affecting emissions from rice include water depth and soil temperature as well as farming practices such as the application of fertilizers and organic matter (Bachelet

and Neue, 1993; Delwiche and Cicerone, 1993; Bodelier *et al.*, 2000) and the presence of alternate electron acceptors such as NO_3^-, Fe^{3+}, and SO_4^{2-} (Bodegom and Stams, 1999).

Sources	Tg/yr	Sinks	Tg/yr
Natural		*Atmosphere*	
Wetlands	115 (55-150)	Tropospheric OH	445 (360-530)
Other	45 (25-140)	Stratospheric OH	40 (32-48)
Anthropogenic			
Fossil sources	100 (70-120)	*Soils*	30 (15-45)
Enteric fermentation	85 (65-100)		
Rice paddies	60 (20-100)		
Biomass burning	40 (20-80)		
Landfills	40 (20-70)		
other	50 (35-110)		
Total Source	**535 (410-660)**	**Total Sink**	**515 (430-600)**

Table 1. Estimated sources and sinks of CH_4 (Tg/year) (from IPCC 1995)

Additional biospheric sources include termites and oceans and anthropogenic sources such as enteric fermentation (primarily from cattle), where CH_4 is produced by MPB in the rumen of animals, biomass burning and landfills. Extraction and combustion of fossil fuels is also a considerable source of CH_4 (around 100 Tg/year) however further discussion of these sources is beyond the scope of this thesis. In terms of sinks the reaction with tropospheric OH accounts for removal of nearly all of the CH_4 produced although

oxidation of CH_4 by methanotrophic bacteria in aerobic soils, also presents a significant contribution to removal of atmospheric CH_4 (IPCC, 1995).

1.5 The wetland methane source

1.51 The global source strength

Wetlands contain up to a third of the world's soil carbon even though these systems cover a mere 5% of the earth's surface (Gorham, 1991). Wetlands are able to retain so much carbon as rates of decomposition are extremely low since lack of oxygen in waterlogged soils stifles oxygen-dependent heterotrophic processes. It is now thought that carbon liberation from wetlands soils is prevented due to oxygen limitation on a single enzyme, phenol oxidase (Freeman *et al.*, in press).

Estimates of the wetland contribution to the global CH_4 budget have varied substantially between 90 and 260 Tg CH_4, or between 17 and 50% of the total source (Matthews and Fung, 1987; Aselmann and Crutzen, 1989; Chappellaz *et al.*, 1993; Bartlett and Harriss, 1993; IPCC 1995; Hein *et al.*, 1997; Cao *et al.*, 1998; Walter, 1998; Houweling *et al.*, 2000). These estimates have been derived by a variety of approaches. They include global extrapolation, or up-scaling, of field measurements using global data sets of wetland area extent (Matthews et al., 1987; Aselman and Crutzen, 1989; Bartlett and Harriss, 1993; IPCC 1995) as well as inverse, or top down, methods where stable isotopes and global monitoring networks are used to constrain budgets (Hein *et al.*, 1997). More recently process-based climate sensitive models of methane emissions have been employed (Cao *et al.*, 1998; Walter, 1998; Walter and Heimann, 2000). The modelling effort by Walter (1998) was validated against CH_4 measurements from six wetland sites spanning

climatically distinct regions and yielded a net wetland emission of 260 Tg yr^{-1}, nearly twice as much as previous estimates. It is thought that this may be an over estimate partly due to validating data sets being from unusually high emitting wetlands (Walter *pers. comm.*).

Houweling *et al.* (2000) simulated pre-industrial atmospheric CH_4 using ice core data of preindustrial mixing ratios and a 3 dimensional chemical transport model. They estimated the CH_4 source strength of natural wetlands as 163 Tg yr^{-1} with a range between 130 and 194 Tg yr^{-1} (Houweling et al. 2000). They suggest that anthropogenic destruction of wetlands may have reduced this source by around 10% although it is possible that warming since pre-industrial times may have negated this decrease (Walter, *pers. comm.*).

1.52 The microbiology of methanogenesis

Methane is produced by a group of micro-organisms known as methanogens (or methane-producing bacteria, MPB) under strict absence of oxygen and reducing conditions of less than –200 mV (Conrad, 1989). Methanogenesis forms the terminal and lowest energy-yielding step of carbon degradation in anaerobic systems, after other electron acceptors such as NO_3^-, Fe^{3+}, and SO_4^{2-} have been consumed (figure 1.3; Conrad, 1989). In such methanogenic systems the ultimate conversion of organic matter to CH_4 and CO_2, as described in equation 1.1 (Conrad, 1989), provides a mere 15 % of the energy that would otherwise be provided during aerobic decomposition (equation 1.2; Schink, 1997).

$$C_6H_{12}O_6 \rightarrow 3\ CO_2 + 3\ CH_4 \quad (\Delta G^{o\prime} = -390\ kJ\ mol^{-1}) \qquad (1.1)$$

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 3\ CO_2 + 6\ H_2O \quad (\Delta G^{o\prime} = -2{,}870\ kJ\ mol^{-1}) \qquad (1.2)$$

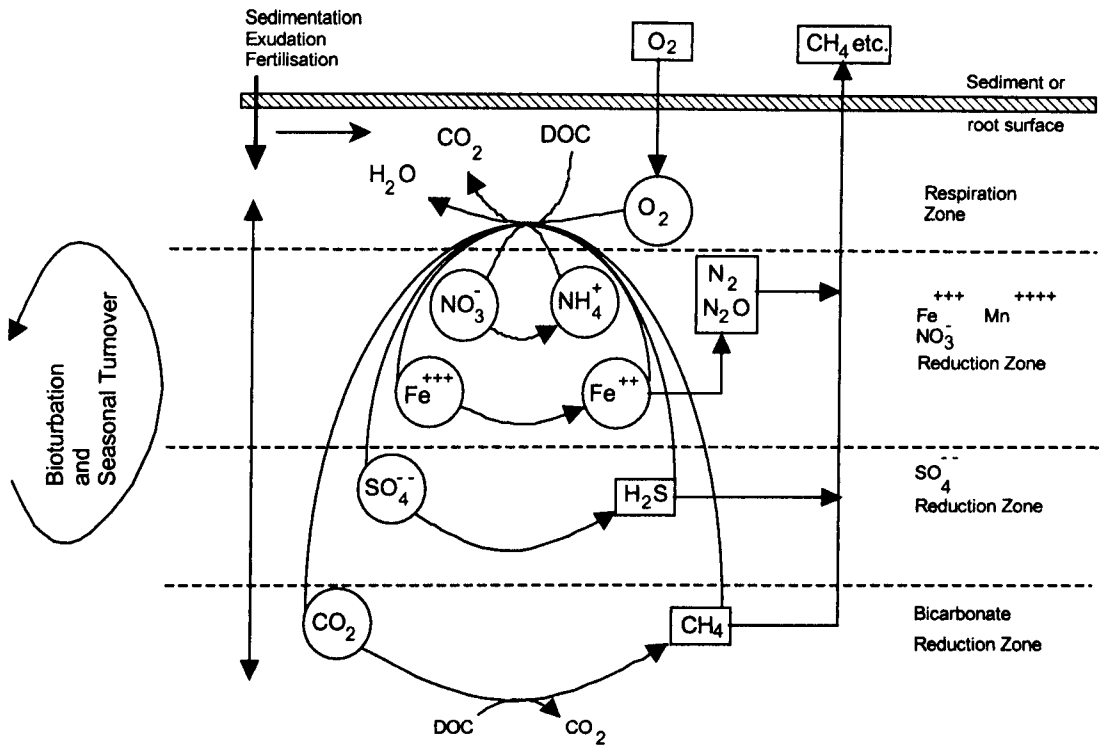

Figure 1.3: Structure of methanogenic systems with respect to redox zonation, regeneration of inorganic electron acceptors and the role of dissolved organic carbon (DOC) (Conrad, 1989)

This conversion of organic matter cannot, however, be carried out by methanogens alone since they are only capable of consuming a narrow range of substrates. There are only three known classes of substrates which methanogens are able to utilize: 1) CO_2, formate (HCOOH) and carbon monoxide (CO) with H_2 providing electrons; 2) methyl group (CH_3) compounds such as methanol and methylamine and 3) acetate (CH_3COOH) (Fenchel and Finlay, 1995). As organic carbon, in its undegraded form, cannot be directly used by methanogens, methanogens are reliant upon other micro-organisms for the provision of available substrates. Conrad (1989) summarised the requisite bacteria for complete degradation of organic matter in anaerobic systems as a) hydrolytic and fermenting bacteria, b) H^+ reducing bacteria, c) homoacetogenic bacteria and finally d) methanogens. The majority of methanogens are able to utilise CO_2/H_2 as a substrate, however the most

common methanogenic substrate in nature is acetate (Oremland, 1988). These substrates provide the major methanogenic pathways in peatlands with H_2/CO_2 contributing around 33% and acetate providing 66% of methanogenesis (Conrad, 1999). The relative contribution of each pathway to emitted CH_4 has been shown to vary seasonally (Kelly *et al.*, 1992; Avery *et al.*, 1999) with acetate more common during the warm growing season and H_2/CO_2 dominating during the winter.

H_2 consumption by methanogens also presents a regulatory mechanism through which excessive production of H_2 by bacteria during early carbon degradation steps can be regulated (Conrad, 1989). Such "syntrophic" cooperation through "interspecies H_2 transfer" between metabolically different microbial groups is essential for the degradation of organic matter, as too high a partial pressure of H_2 can prove detrimental to both fermenting and fatty acid oxidising bacteria (Schink, 1997). For further discussion of syntrophic cooperation in organic rich methanogenic systems see section 1.8.

1.53 Factors controlling CH_4 emissions from peatlands

As discussed in the previous section, anaerobiosis is the primary requirement for methanogens to exist and produce CH_4. Once anaerobic conditions occur, various factors may affect a) the rate at which CH_4 is produced and b) the rate at which the CH_4 is consumed by aerobic methane-consuming (or methanotrophic) bacteria. In peatlands, the three main variables affecting these processes are water table, temperature and substrate quality (Dise *et al.*, 1993; Nilsson and Bohlin, 1993; Moore and Dalva, 1993; Daulat and Clymo, 1998; Macdonald *et al.*, 1998). The depth of the water table determines the proportion of the peat column that is under anaerobic conditions and so defines a zone of potential methanogenesis (Lloyd *et al.*, 1998; Daulat and Clymo, 1998). Substrates derived from plant material, either from root exudates or decomposing plant litter, are

easily degraded and eventually consumed by methanogens (Conrad, 1989). The requirement for methanogens of proximity to labile carbon sources coupled with the necessity of distance from the O_2 source (surface) means that the zone of maximum methanogenesis is located approximately 10 cm below the water table (Sundh *et al.*, 1994). Above this water saturated zone, oxygen availability limits methanogenesis and permits methanotrophic bacteria to consume CH_4 that was produced in the lower anaerobic zone (Roslev and King, 1996). The difference between CH_4 production and consumption, therefore, determines the net emission of CH_4 from wetland soils (Sundh *et al.*, 1994). In addition temperature controls the rate at which both CH_4 production and consumption takes place with observed Q_{10} values of between 1.7 and 16 for CH_4 production and between 1.4 and 2.1 for CH_4 oxidation (reviewed in Walter, 1998)

The means by which CH_4 is transported from the anoxic zone to the surface is a strong controlling variable in determining net CH_4 emissions. The three different transport mechanisms are diffusion, ebullition and plant-mediated transport. The transport mechanism determines both the rapidity with which CH_4 is transported from the zone of production, as well as the rate at which CH_4 is oxidised. Diffusion of CH_4 is slow through saturated soils and, although CH_4 diffuses faster in unsaturated soils, it is also more susceptible to consumption by methanotrophic bacteria. Consequently diffusion represents a small fraction of the total CH_4 flux from peatlands (Conrad, 1989). Ebullition of bubbles containing a combination of CH_4 and CO_2 may form in wetland soils due to the low solubility of CH_4 in these systems. These form a rapid and sporadic means of CH_4 emission, which, while generally contributing little to peatland effluxes, may enhance emission during periods of low atmospheric pressure (Mattson and Likens, 1990).

The dominant form of transport of CH_4 from the site of production to the atmosphere is via vascular plant tissue (Schimel, 1995; Shannon *et al.*, 1996; MacDonald *et al.*, 1998). Aerenchymal tissue in plants allows O_2 to descend to the plant roots, thereby allowing them to respire in an otherwise anaerobic environment. This same mechanism permits dissolved gasses in the peat to escape to the atmosphere, thus bypassing the zone of maximum methane oxidation potential. As a result, this transport pathway is responsible for up to 90% of CH_4 emission from peatlands (Shannon *et al.*, 1996).

1.6 Atmospheric sulfur pollution

The role that sulfur may play in regulating the radiative balance of the earth's atmosphere has been the subject of much recent study (Charlson *et al.*, 1991; Hanson *et al.*, 1998 and references therein). In the atmosphere, sulfur as aerosols can directly affect the earth's radiative balance by increasing the regional albedo i.e. reflecting incoming solar radiation back to space (IPCC, 1995). Atmospheric sulfates also affect radiative balance indirectly by providing additional cloud condensation nuclei and, in doing so, increasing the albedo of clouds by increasing the water droplet concentration (IPCC, 1995). Should increases in S deposition affect the output of radiatively important CH_4 from impacted wetlands, this will present an additional and as yet un-characterised climate feedback.

It is thought that the total northern hemispheric anthropogenic flux of sulfur first exceeded natural emissions during the early 20th century (Rodhe, 1999) and in the mid 1990's global anthropogenic emissions exceeded natural emissions by a factor of three (Rodhe *et al.*, 1995). Up to the end of the first half of the 20th century, the emission and deposition of oxidised sulfur pollution, primarily as a result of coal burning, was largely confined to urban areas. This local problem was reduced through a variety of strategies, which in

Britain were implemented through the Clean Air Acts of 1956 and 1968 (RGAR, 1997). These successful strategies included the location of large sources, such as power stations at remote locations away from urban areas as well as increasing the height of chimneys. The unforeseen consequence of these actions, however, was to increase the potential for long-range transport of acidifying S species thereby impacting pristine environments, including wetland areas, many hundreds to thousands of kilometres away from pollutant sources (RGAR, 1997). Rapidly increasing rates of deposition during the 1960's and 70's in Scandinavia and central Europe reflected this enhanced long-range transport (Mylona 1996). Thereafter international, transboundary protocols were enacted (e.g. the Helsinki Protocol or 30% club) to decrease oxidised sulfur emissions (RGAR, 1997). As a result anthropogenic S emissions in Europe and the western region of the Former Soviet Union have decreased by 55% between 1980 and 1996 (Rodhe, 1999).

While in United States and Europe trends of decreasing S emissions/deposition have characterised the last 2-3 decades, in other regions of the world the situation is very different (Rodhe, 1999). Asia in particular is experiencing rapid economic growth and industrialization which, together with a growing use of indigenous coal as the primary regional fuel source, is resulting in increasing emissions of sulfur (Arndt and Carmichael 1995). While S pollution has generally been a local problem in Asia, continued growth and coal consumption is forecast to have regional consequences, with potential for long-range transport to affect pristine environments such as Siberia and Indonesia, regions of extensive CH_4 emitting peatlands (Bhatti *et al.*, 1992).

1.7 Sulfur dynamics in wetlands

Peatlands are generally nutrient-poor systems where entire nutritional inputs are derived from atmospheric inputs. As such, peatlands may be affected by long-range transport of pollutants such as S and N species (Gorham *et al.*, 1984) and so the peatland cycling of these pollutants is likely to be affected (Wieder *et al.*, 1987). Brown and Macqueen (1985) found that peatlands are net sinks of S. Through applying $^{35}SO_4^{2-}$ (a radioactive tracer which allows the pathways of SO_4^{2-} in anaerobic soils to be examined) they showed that only a small fraction of applied SO_4^{2-} remained in this water-soluble form (2.2%) with 11% being reduced via dissimilatory SO_4^{2-} reduction processes (see section 1.8) to an "acid-volatile" inorganic sulfur pool that consists of H_2S and FeS with the remainder incorporated as organic matter (as ester sulfates and as carbon bonded S). Wieder *et al.*, (1990) investigated S-cycling in two Appalachian peat bogs and interpret their findings as suggesting that SO_4^{2-} reduction, at rates in excess of those which might be expected due to SO_4^{2-} inputs from acid rain alone, may be the dominant means of carbon flow in peatlands. They support this with the hypothesis that high rates of SO_4^{2-} reduction (equivalent to those reported for SO_4^{2-} rich coastal marine sediments) may be maintained through rapid turnover in the SO_4^{2-} pool through continuous oxidation and reduction of reduced S species. Although the findings by Brown and MacQueen (1985), imply that over a short period of time the majority of available SO_4^{2-} is eventually added to the organic S pool, recent findings by Groscheova *et al.* (2000) using ratios of naturally abundant stable S isotopes, imply that the available SO_4^{2-} pool may be replenished via hydrolysis of ester SO_4^{2-} in addition to reoxidative processes involving sulfides in the inorganic S pool (Weider and Lang, 1988).

1.8 Microbial interactions between sulfate-reducers and methanogens

In anaerobic organic systems, a variety of different microbial populations exist, working in a complex food web that allows the ultimate degradation of organic material to CH_4 and CO_2. The presence of alternate electron acceptors such as O_2, NO_3^- and SO_4^{2-} in anaerobic sediments and soils can, however, directly affect the production CH_4, shifting the terminal degradation step away from CH_4 production in favour of increased CO_2 production (Conrad, 1989). These oxidised inorganic species are utilised in a thermodynamic sequence that decreases with distance from the oxic/anoxic interface (fig 1.3). Sulfate reduction provides a means of completion of this degradative sequence and yields more energy for sulfate-reducing bacteria (SRB) than does the production of CH_4 by methanogens that occupy similarly low redox niches (Ward and Winfrey 1985).

Consequently much work in recent decades has focused on the interaction between methanogens and SRB as influenced by SO_4^{2-} supply and substrate availability for a variety of environments ranging from ocean and esturine sediments and freshwater sediments and soils, to engineered reactor systems, rice-growing systems and the human gut (Ward and Winfrey 1985; Conrad et al., 1987; Widdel, 1988; Conrad, 1989; Raskin *et al.*, 1996).

In SO_4^{2-}-rich natural environments, SRB are generally competitively superior to methanogens over mutual substrates such as hydrogen and acetate (Abram and Nedwell 1978). In freshwater systems the opposite was thought to be true with methanogens out-competing SRB for hydrogen and acetate (Ward and Winfrey, 1985) although Lovley and Klug (1983) found that freshwater concentrations of SO_4^{2-} may be sufficient to stimulate SRB into outcompeting methanogens. Findings during the 1980s and 1990s, however,

suggest that this established model may be an over simplification of the processes operating in fresh water environments (Conrad *et al.*, 1987; Scholten and Stams, 1995; Raskin *et al.*, 1996; Watson and Nedwell, 1998). These studies suggest that, while methanogens may be dependent on other microbes for their supply of substrates (e.g.H_2), these microbes may themselves require the methanogens in order to maintain suitably low partial pressures of H_2, which would otherwise increase to harmful levels. Such "syntrophic" associations have been observed in low SO_4^{2-} fresh water systems with SRB implicated in the degradation of higher chain fatty acids while providing H_2 through "interspecies H_2 transfer" for syntrophic methanogenic partners (Conrad *et al.*, 1987; Raskin *et al.*, 1996).

Conrad *et al.*, (1987), working with eutrophic lake sediments (Lake Mendota USA) interpreted the immediate inhibition of H_2-dependent methanogenesis upon SO_4^{2-}addition as indicating the pre-existence of SRB in a portion of the H_2-syntrophic microbial associations existing in the sediments. This finding is at odds with the established paradigm of mutual exclusivity between SRB and methanogens in SO_4^{2-}-depleted sediments.

Evidence has grown that syntrophic consortia, which may include SRB despite low SO_4^{2-} concentrations, may contribute an efficient means of terminal carbon degradation in freshwater systems. Tatton *et al.* (1989) were able to maintain a continuous, stable methane-producing culture of three bacteria types, a SRB (*Desulfovibrio*) and two types of methanogen, in a SO_4^{2-} free medium, with close proximity of the different bacteria types in flocs benefiting the individual bacteria by improving interspecies H_2 transfer efficiency. Indeed, Conrad *et al.* (1985) were able to show that only 5% of H_2 dependent

methanogenesis was maintained by H_2 available in the bulk H_2 pool and so H_2 within microbial aggregates must be the dominant source for methanogenesis.

Raskin *et al.* (1996) were further able to investigate dynamics of natural anaerobic microbial communities under varying SO_4^{2-} status by growing anaerobic biofilms derived from a ground-water filtration innoculum in either methanogenic or sulfidogenic reactors. Using phylogenetic probes they found that methanogens and the dominant SRB comprised 25% and 16% respectively, of microbial communities in methanogenic reactors when theoretically there was only sufficient SO_4^{2-} to support SRB at a proportion of 1% of the total microbial community (Raskin *et al.*, 1996). When SO_4^{2-} concentrations were increased in the methanogenic reactors they found that the relative numbers of SRB increased, with the dominant SRB increasing to 26% after around 20 days, then decreasing to minimum levels of 20% after a period of 50 days before reaching a steady state of around 35% after 100 days (Raskin *et al.* 1996). Following SO_4^{2-} addition, methane concentrations in reactor effluent immediately decreased to below detection limits which they interpret as resulting from existing SRB being able to immediately out-compete methanogens for mutual substrates (Raskin *et al.* 1996). It is also possible that a switch to sulfate reduction in SRB from a previous "SO_4^{2-} deprived" function of syntrophically degrading carbon with associated methanogens may have deprived the methanogens of an important H_2 or acetate source (Conrad *et al.* 1987). Methanogen numbers were found to decrease slowly to a new steady state level of 8% of the total microbial population after several months of elevated SO_4^{2-} concentrations in reactor influent (Raskin *et al.*, 1996).

In peatland systems there has only been limited research on these interactions (Nedwell and Watson, 1995; Watson and Nedwell, 1998). Nedwell and Watson (1995) found that the addition of low concentrations of SO_4^{2-} to homogenised peat samples (around 500 μM)

significantly decreased the rate of methane production. Interestingly, the same occurred with the addition of a specific SRB inhibitor (molybdate) (Watson and Nedwell, 1998) which was interpreted to mean that SRB were actively involved in methanogenesis, as was found for sediments in the Lake Mendota study (Conrad *et al.*, 1987). Watson and Nedwell, (1998) also found that the proportion of carbon flowing through either methanogenesis or SO_4^{2-} reduction in Ellergower Moss was dependent on seasonal temperature changes with SO_4^{2-} reduction favoured during winter (up to 99.9% of carbon flowing via this pathway). During the summer, SO_4^{2-} reduction accounted for less than 30% of carbon flow, with methane production dominating as the terminal means of carbon degradation (Watson and Nedwell, 1998). Watson and Nedwell, (1998) suggest this shift in carbon flow is determined by SO_4^{2-} concentrations. This does not, however, explain why the ratio of SO_4^{2-} reduction to methane production was 20 times larger in winter than in spring when the highest SO_4^{2-} concentrations were measured (Nedwell and Watson 1995; Watson and Newell, 1998). An alternative explanation may be that competition favouring SRB over methanogens may not be inevitable, and may in fact be dependent on temperature. By this scenario cool winter temperatures favour SO_4^{2-} reducers over methanogens when H_2/CO_2 is the major methanogenic pathway, and warm periods may favour methanogens over SRB when competing over acetate (the dominant methanogenic pathway in summer (Kelly et al., 1992; Avery et al., 1999; Bodegom and Stams, 1999).

1.9 SO_4^{2-} manipulation experiments in freshwater wetland systems.

Although the evidence discussed thus far suggests that elevated supply of SO_4^{2-} to peatlands from acid rain may significantly reduce CH_4 emissions, very few studies have examined this likely interaction by manipulating inputs of SO_4^{2-} to peatlands and

measuring for possible effects on net methane flux (Fowler *et al.*, 1995; MacDonald 1998; Dise and Verry 2001.). There have been more experimental manipulations of SO_4^{2-} input to rice paddy systems while measuring CH_4 emissions (Lindau *et al.*, 1994; Denier van der Gon and Neue 1994; Lindau *et al*, 1998). These-rice based experiments have demonstrated that the addition of gypsum ($CaSO_4$) of the order of 10^3 kg ha^{-1} reduces methane emissions by between 29% and 46% for applications of 1,000 and 2,000 kg $CaSO_4$ ha^{-1} (Lindau *et al.*, 1994) and by 55% to 70% for application rates of 6,660 kg $CaSO_4$ ha^{-1} (Denier van der Gon and Neue 1994) a range of suppression also reported by Lindau *et al.*, (1998).

Fowler *et al.*, (1995) found that a single application of SO_4^{2-} at a rate of 100 kg S ha^{-1} to peat monoliths (approximately twice the annual input of SO_4^{2-} from acid rain to peatlands in the Pennine Hills), resulted in CH_4 suppression of approximately 50% within two weeks of the application, a suppression within the range of observed suppression in rice paddies with SO_4^{2-} application an order of magnitude smaller. Unpublished data by Fowler *et al.*, with more representative SO_4^{2-} applications of around 40 kg S ha^{-1} resulted in a similar suppressive effect although they found that two months after the SO_4^{2-} application, methane emissions had recovered to pre-treatment levels. This has lead to speculation that large, individual applications of SO_4^{2-} may stimulate a boom followed by a crash in SRB populations as SO_4^{2-} is consumed and converted to reduced sulfur forms (Arah and Stephen, 1998; Dise and Verry, 2001). It has, however, been shown that sulfides may be reoxidised in aerobic surface layers of the peat column, continually replenishing the dissolved SO_4^{2-} pool and facilitating the maintenance of larger sulfate reduction rates than instantaneous SO_4^{2-}concentrations would otherwise suggest, and, in doing so, maintaining the competitive disadvantage of methanogenic populations (Wieder *et al.*, 1990; Freeman *et al.*, 1994). An alternative explanation may be that microbial communities experiencing

elevated SO_4^{2-} concentrations, having previously experienced far smaller concentrations of SO_4^{2-}, may go through a period of 'readjustment' where SRB populations increase and then decline to levels close to those of the pre-treatment period before again increasing to an enlarged, and methanogenesis inhibiting, steady state population (Raskin *et al.*, 1996). Indeed this fluctuating population of SRB observed by Raskin *et al.*, (1996) during the initial stages of an enhanced SO_4^{2-} perturbation, reflects well the CH_4 flux suppression and subsequent recovery observed by Fowler *et al.*, (unpublished data) both in terms of the direction and timing of observed changes.

Fewer studies have investigated the effects of the prolonged addition of frequent small doses of SO_4^{2-} that may more closely replicate the mode of sulfur deposition experienced in acid rain-impacted areas. Only one experiment investigating the effect of such frequent, small SO_4^{2-} applications had taken place by the time the work reported in this thesis had begun (Dise and Verry, 2001). Dise and Verry (2001) examined the effect of weekly pulses of 2.7 kg of SO_4^{2-} -S ha^{-1} over a, 9-week period during the growing season. Although the overall rate of application (145 kg $ha^{-1}yr^{-1}$) was similar to previous manipulation experiments by Fowler *et al.* (1995), individual pulses were an order of magnitude smaller. CH_4 fluxes were reduced by 37%, again within the range of suppression experienced in soils manipulated with far larger SO_4^{2-} applications.

1.10 Thesis aims and layout

The apparent lack of response in CH_4 flux suppression to either the size or rate of SO_4^{2-} application within the range applied in the literature reviewed above, suggests that far lower rates of SO_4^{2-} deposition, corresponding to those experienced in many industrialised and developing regions, may significantly impact carbon flow in peatlands and ultimately,

CH_4 fluxes from the largest individual source of atmospheric CH_4. The principal aim of this investigation, therefore, was to remedy this deficiency in our understanding by replicating a range of commonly-experienced SO_4^{2-} deposition rates of acid rain impacted regions in a pristine natural peatland in northern Scotland while monitoring CH_4 emissions. Additional environmental variables (i.e. temperature and water-table) were also monitored in order that the major factors controlling any suppressive effects of SO_4^{2-} on CH_4 fluxes could be quantified. Chapter 2 presents the principal methods used throughout the thesis and gives a description of the field sites used. Additional methods of a more specific nature to the different investigations presented are reported in the individual chapters. The details of this two-year field manipulation experiment are presented in Chapter 3, which is largely in the form of a scientific journal article and has been submitted for publication to the journal *Global Biogeochemical Cycles*.

Chapter 4 details an investigation of the processes within the experimentally manipulated peatland and explores how they may have been affected by enhanced SO_4^{2-} deposition. Chapter 5 presents a process-based study of the effects of enhanced SO_4^{2-} and the possible effect of temperature on CH_4 emissions from SO_4^{2-} impacted peat monoliths maintained within controlled environment conditions. In this chapter investigations of the effect of SO_4^{2-} application mode (i.e. single doses or continuous small additions of SO_4^{2-}) are also presented as is an analysis of how the different sulfur pools may have been affected.

In Chapter 6, in collaboration with workers from the National Aeronautics and Space Administration, Goddard Institute for Space Studies (NASA GISS) USA, a global scale view is taken by applying the findings of Chapters 3 and 5 to newly developed global estimates of CH_4 emissions from wetlands derived from an adaptation of climate sensitive process based model (Walter, 1998; Walter and Heimann, 2000). This is in combination

with modelled deposition estimates of sulfur from tropospheric S simulations in a chemical transport model (Koch *et al.*, 1999). As such estimates are provided of the global impact changes in sulfur deposition may have on the wetland CH_4 source for a variety of scenarios spanning the second half of the 20th Century up to the late 21st Century.

Chapter 7 presents a general discussion of the combined findings presented in the various investigations and summarises the main conclusions of the study.

Chapter Two

Materials and Methods

2.1 Introduction

In this chapter, the main methods used to investigate the effect of SO_4^{2-} deposition at acid rain rates, on CH_4 fluxes from a peatland in northeast Scotland and from peat monoliths collected from Caithness in northern Scotland are detailed. A detailed description of the field site that formed the main focus of this study is also provided. In addition, the various methods available for the measurement of CH_4 fluxes are discussed.

2.2 Experimental site description

A long-term SO_4^{2-} manipulation and CH_4 monitoring experiment was established on a pristine portion (i.e. unaffected by cutting or drainage) of a peatland known as Moidach More in Morayshire in the northeast of Scotland (57.46 N, 3.62 W). The peatland is probably the largest individual mire in northeast Scotland and, while representing the range of peatland features found throughout Britain, also demonstrates similarities in vegetation to boreal mires found in Scandinavia (Lindsay 1992).

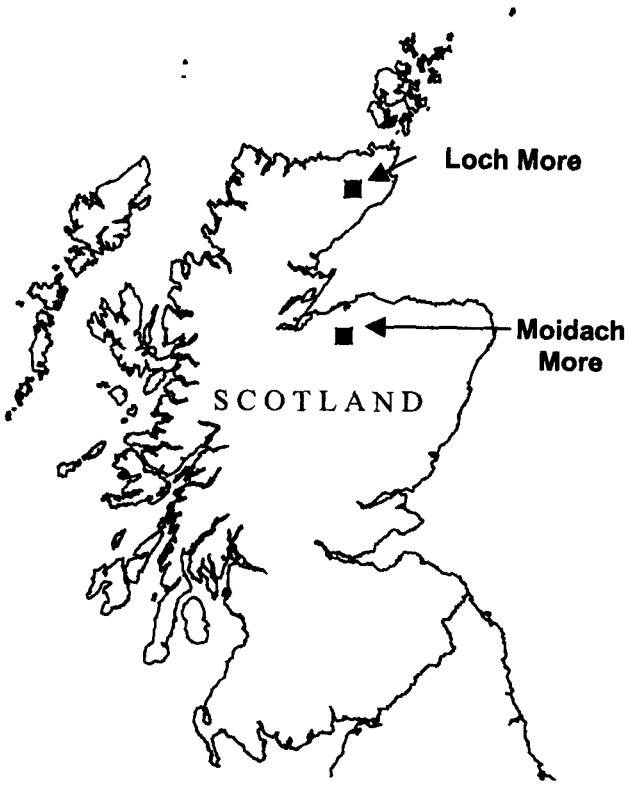

Figure 2.1: Location of the two peatlands used in this study (see section 2.6 for description of Loch More).

The peatland is situated at an altitude of 275 m above sea level. It consists of peat of more than 0.5m thick extending over 760 ha and averaging 2.1m in depth. The mean annual precipitation at the site is approximately 900 mm (Meteorological Office 1987) and the mean annual temperature is 8°C (Williams *et al.* 1999). The vegetation mainly comprises *Sphagnum* species, which include *S. magillanicum* and *S. capillifollium* (Ehrh.) Hedw. and *S. revurvum* (P.Beauv.). The dominant sedge is *Trichophorum cespitosum* (L.) Hartm. (Deer Grass). Other plants include *Erica tetralix* L. and, in areas of the bog affected through cutting and burning, *Calluna vulgaris* (L.). The site was selected for its low ambient SO_4^{2-}-S deposition rate of around 5 kg $ha^{-1}year^{-1}$ (R.Smith pers. comm.). Rainfall data for the area were collected at Grantown on Spey, approximately 10km south of the study site (supplied by the British Atmospheric Data Centre).

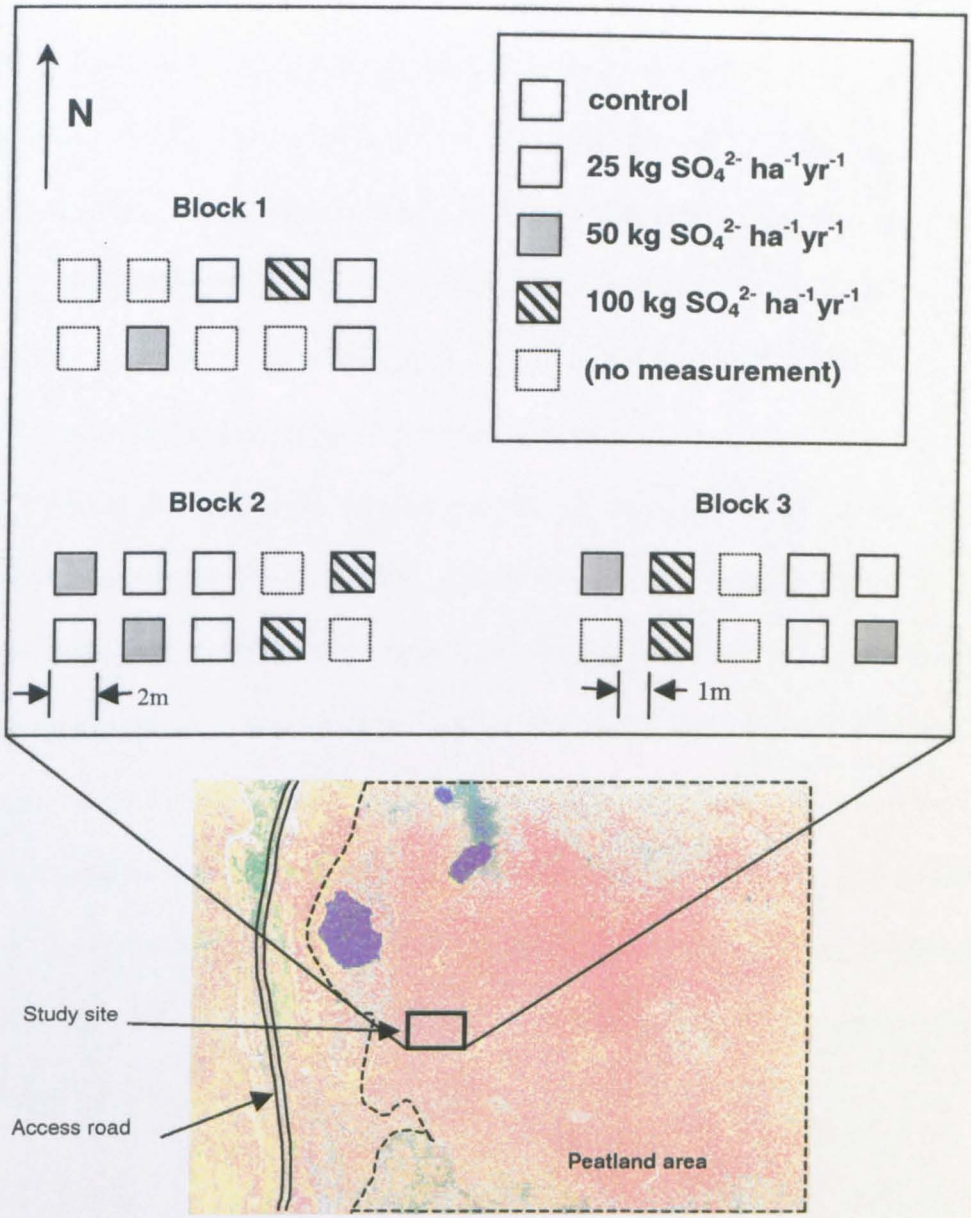

Figure 2.2: Field site arrangement for the investigation of the effects of different SO_4^{2-} deposition rates on CH_4 emissions (Moidach More)

2.3 Experimental Manipulations at Moidach More

Wooden boardwalks were installed within the sampling area to allow repeated access while minimizing site disturbance during sampling. 20 experimental plots (2 x 2m) were established on an area of the peatland that exhibited uniform characteristics in terms of its vegetation, topography and hydrology (fig 2.2). The plots were separated

by a 1m buffer strip and were randomly assigned to one of three treatments or a control. The treatments consisted of additions of 20, 45 and 95 kg SO_4^{2-}-S per hectare per year applied as Na_2SO_4. In addition to annual ambient deposition, this amounted to total annual deposition rates of 25, 50, and 100kg SO_4^{2-}-S ha^{-1} respectively. The treatments were applied as weekly doses of between 1.2 and 4.7 mmol SO_4^{2-}-S in 1 litre of deionized water, which amounted to between 0.5 and 1.9 kg SO_4^{2-} -S ha^{-1} wk^{-1}. Over the winter period (November to March) applications were made once a month rather than weekly, and doses were correspondingly four times stronger in concentration. SO_4^{2-} was added as Na_2SO_4 (as opposed to H_2SO_4) to minimise any potentially confounding pH effects. Weekly doses of Na_2SO_4 in 1 litre of deionized water were sprayed evenly onto each 2 x 2m plot using a pressurized garden sprayer (Hozelock, UK). The solution added amounted to a hydrologically negligible weekly increase of 0.25mm of water to the system. Controls received the same volume of deionized water. All experimental additions began on 25th June 1997 following five weeks of CH_4 flux monitoring at the site.

2.4 Methane flux measurements

2.4.1 Introduction

A variety of methods are available for the measurement of trace gas emissions (in this case CH_4), from soils. The two broad categories in which they fall are enclosure, or chamber methods and micrometeorological techniques. Both types of technique have their own advantages, depending on the information being sought from the investigation. Soil enclosure or chamber techniques are routinely used to measure trace

gas fluxes (CO_2, CO, CH_4, N_2O, NO, etc.) and have a number of advantages over micrometeorological methods (Mosier 1989). These advantages include their suitability for determination of small fluxes, which may be below the detection limits of micrometeorological methods; they are relatively inexpensive to make and can be used in a wide variety of environments. A key attribute, which determined their use in this study, is that chambers are an ideal method for the measurement of fluxes from systems that are being experimentally manipulated in small-scale experiments. Chambers, however, are not without problems and these are discussed in section 2.4.2.

Micrometeorological methods offer a different set of advantages over chamber techniques. A key advantage is that fluxes may be measured over large areas and, as a result may avert problems of small–scale spatial heterogeneity that may be encountered by chamber methods. In addition micrometeorological techniques do not introduce the possibility of site disturbance that may occur through chamber methods. For a full review of micrometeorological methods see Fowler and Duyzer (1989).

2.4.2 Chamber methods, associated problems and their minimisation.

There are two basic chamber types, the first, known as closed or 'static' chambers, involves the calculation of flux by periodically taking samples from within a defined chamber 'head space' and then measuring the rate of change in gas concentration during the period of linear concentration change. The second chamber type is the open or 'dynamic' chamber. This method works by allowing a continuous flow of air from the atmosphere into the chamber via an air inlet, over an area of soil that is defined by the chamber. The air then leaves the chamber via an outlet and the flux is calculated from

the change in concentration between the outlet and the inlet to the chamber, the chamber area and the flow rate. The main advantage of this method is that conditions experienced naturally can be better approximated. A disadvantage, however, is that measurement error can be easily introduced through changes in pressure within the chamber which can lead to underestimation or overestimation of the natural rate of gas flux. In addition, the measurement system requires electricity and air pumps, a requirement that made this method unsuitable for the main field study reported here (Chapter 3). For a detailed review of chamber methods see Mosier (1989).

Taking these factors into consideration, static, closed chambers were chosen for use in both CH_4 flux studies reported in this thesis (Chapters 3 and 5). It is important, however, that sources of error associated with this measurement technique are discussed as well as the measures that were taken in this study in order to minimise them.

Site Disturbance

There is potential, when inserting chambers into soils, that the chamber may effect the processes or transport mechanisms that determine rates of emission for the gas under investigation. This can occur through in a variety of ways. Compaction in peat soils when chambers are inserted can have the temporary effect of increasing fluxes while if roots of plants within the chamber area are severed, vascular plant transport of CH_4, may be reduced. This was of minimal importance in the controlled environment monolith study (section 2.6 and Chapter 5) as chambers were at no time placed on the peat surface. In the long-term study at Moidach More (section 2.5 and Chapter 3), this potential source of error was minimised by using a cutting knife to facilitate insertion of

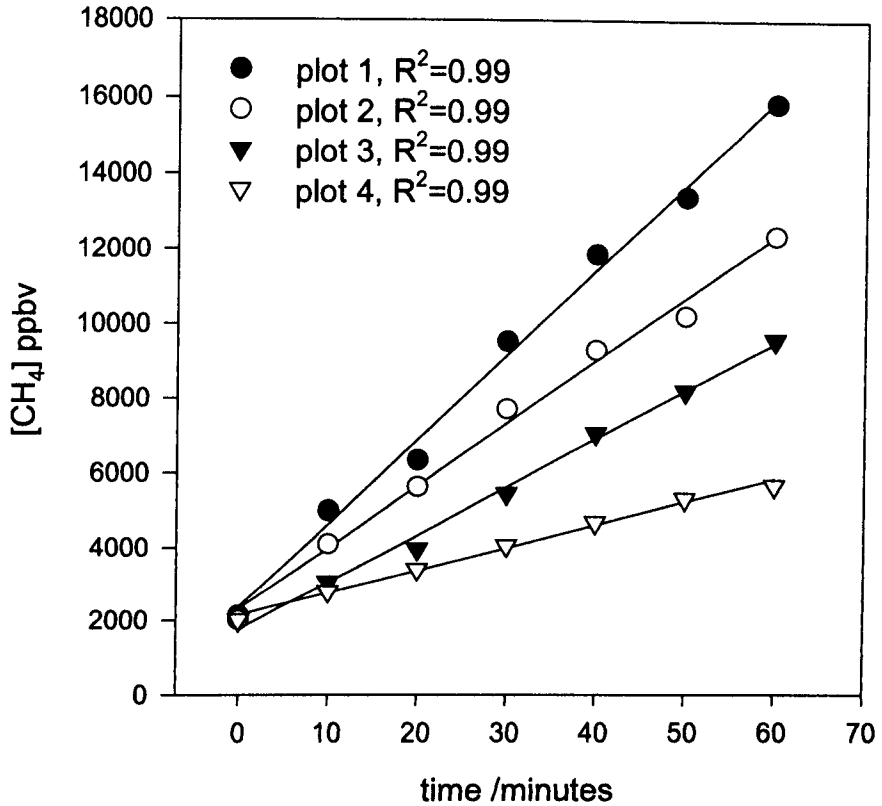

Figure 2.3: Experiment showing linear increase in chamber headspace CH_4 concentrations with time for four experimental plots.

collars into the peat, which could then be repeatedly sampled through the addition of a lid, which defined a chamber headspace.

Concentration Effects

Since diffusion is an important mechanism with which CH_4 from peatlands is emitted to the atmosphere (Conrad 1989), and given that this mechanism is dependent on a concentration gradient between the point of production in the point of emission (i.e. the atmosphere) there is potential for increasing concentrations of CH_4 in the headspace to limit further emission via diffusion (Mosier 1989). This was minimised in the Moidach more study by keeping enclosure times to a minimum. Preliminary experiments with

chambers positioned in a selection of plots showed that this effect was insignificant for enclosure times of less than one hour as CH_4 concentration growth within the chamber was linear over this time (fig 2.3).

Temperature effects.

With microbial processes responsible for CH_4 emissions being strongly influenced by temperature, it possible that temperature changes brought about by the chamber affecting radiative balance (i.e. acting as a greenhouse) may increase fluxes. In practice, however, it takes far longer for peat to increase in temperature than air in the chamber volume and it has been found, through using similar methods, that there is no significant increase in peat temperatures over a 1 hour enclosure period (MacDonald, 1998). In the experiments reported in this thesis all samples were taken within one hour of a chamber being closed, more usually around 20 minutes (Moidach More) or 40 minutes (peat monoliths).

Pressure Changes

Enclosure methods may also prevent fluctuations in atmospheric pressure from affecting fluxes within the enclosure. These fluctuations may stimulate emissions by increasing soil air movement and so excluding enclosed air masses from such natural variability may provide an underestimate of real flux rates (Mosier 1989). Inclusion of a vent may alleviate such a problem although no such mechanism was included in this study.

In practice the main purpose of the experiments reported here, was to evaluate the difference in CH_4 fluxes between different experimental SO_4^{2-} manipulations relative to

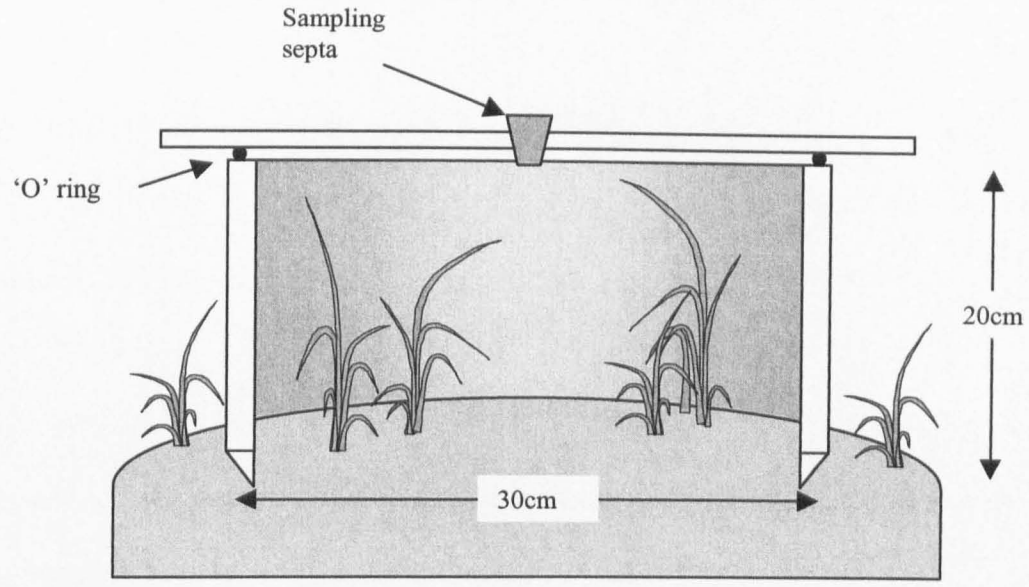

Figure 2.4: Chamber methods used at Moidach More

controls CH_4 emissions where the same methods were used. It is therefore likely that the methods employed in this study (section 2.5) were appropriate means of meeting this objective.

2.5 CH_4 measurements at Moidach More

CH_4 flux was measured using static chambers, which were semi-permanently (for the duration of the experiment) placed within each experimental plot (figure 2.4). The chambers consisted of sections of polypropylene pipe (length 25 cm, internal diameter 30cm). A groove was machine cut into the top edge of each section to accommodate a neoprene 'o' ring. The bottom edge of each section was bevelled to facilitate installation of the chambers to a depth of 5 cm into the peat surface. Once in position the chambers were not moved again for the duration of the experiment.

The headspace volume was defined by placing a transparent acrylic lid onto the 'o' ring. Each lid was fitted with a silicone rubber septum (Suba Seal) which allowed repeated sampling of the headspace gas with a needle and syringe, both immediately after enclosure and then again 20 minutes after the initial sample was taken. Three way stopcocks allowed samples to be stored in the syringes prior to analysis (within 24 hours). Prior to the start of the experiment, all syringes used were tested for leakage by filling them with a 3400 ppbv CH_4 standard. Samples within the syringes were then measured via gas chromatography (see section 2.5.1) both at time = 0 hrs and time = 24 hrs. Syringes where more than 5 % of the concentration was lost during this time were discarded (only one syringe). The average percentage loss of CH_4 was 3% over 24 hrs. Time series experiments, where chambers were enclosed for an hour and sampled repeatedly at 10-minute intervals showed that over this time span the increase in chamber CH_4 concentrations was linear (fig 2.3). At bi-monthly intervals, the headspace of the chambers was sampled repeatedly (a minimum of 3 samples) over an hour-long period to test for linearity in the increase of methane concentration. Each plot was sampled weekly (late spring – early autumn) to monthly (late autumn – early spring) starting in May 1997. Samples were taken within an hour of noon on each sampling day. Chambers were sampled for CH_4 flux prior to SO_4^{2-} applications at all times.

CH_4 fluxes were calculated using the following linear equation:

$$\text{Flux } (CH_4) = \frac{\Delta C \times f \times V}{a \times t} \qquad (2.1)$$

Where ΔC is the change in CH_4 concentration (ppbv), f is a concentration to mass conversion function, V is the volume of the headspace (m^3), a is the soil area as defined

by the chamber (m^2) and t is the enclosure time (minutes). CH_4 fluxes for the long-term field monitoring experiment and the short-term controlled environment experiment are reported with different units. Long-term field based flux measurements using chambers are commonly reported in mg m^{-2} day^{-1} (e.g. Dise, 1993; Dise *et al*, 1993) where as in process-based experiments, fluxes tend to be reported in ng m^{-2} s^{-1} (Macdonald 1998).

2.5.1 Method of analysis.

Gas Chromatography was used throughout this study to measure concentrations of CH_4 in sample syringes. This method relies on the ability of a porous column (stationary phase) to partition different compounds that may be introduced to a carrier gas (mobile phase) which is passing through the column. Individual components within the introduced sample are retarded for different lengths of time depending on the extent of interaction between the component and the column with components emerging in order of increasing interaction with the column.

When CH_4 has emerged from the column it is detected on a flame ionisation detector, where, after first being pyrolysed in a H_2/air flame, resulting ions and electrons allow a current to flow between an electrode and an ion collector. This current is then measured, converted to a digital signal and monitored using a commercial integrator, which calculates the area beneath the curve produced by variations in current with time.

In this study CH_4 concentrations in the syringes were analysed on a Chrompack CP9000 gas chromatograph fitted with a flame ionization detector GC FID with a 2m long Poropack Q column connected to a Spectra Physics integrator. The GC was periodically calibrated against a 1960 ppbv compressed air CH_4 standard and 3400 ppbv

standard (both from Air products, UK). The GC had a precision of less than 1%, which is equivalent to around 20 ppb for the 1960 ppb standard.

2.6 CH_4 measurements from peat monoliths under controlled conditions

Monoliths for the controlled environment study (n = 24, dimensions 40cm by 30cm) were collected in October 1998, using a 30cm diameter aluminium corer, from an area of extensive peat cover adjacent to Loch More, which forms part of the Flow Country in Caithness, Northern Scotland (58° 23' north, 3° 36' west – see fig 2.1). Vegetation within the monoliths comprised *Sphagnum spp.*, and the dominant vascular plants in the monoliths were *Trichophorum cespitosum* and *Eriophorum* spp. The use of peat monoliths in controlled environment conditions has the advantage over the examination of natural in-situ ecosystems in that those variables which control methane fluxes (mainly temperature and water table) can be closely controlled independently of one another. Large monoliths, as used in this experiment, offer the advantage of maintaining the soil and vegetation structure, thereby allowing field conditions to be approximated for process studies.

The monoliths were transported to a glasshouse at CEH Edinburgh where they were maintained at 15°C and were given an extended day (12hrs) through artificial lighting for two months during mid-winter. This treatment was applied in order to bring forward the onset of the growing season as wetland plants play an important role in the emission of methane from peat. The resultant stimulation of early plant growth better reproduced the stage of vegetation during the peak methane-emitting growing season throughout the

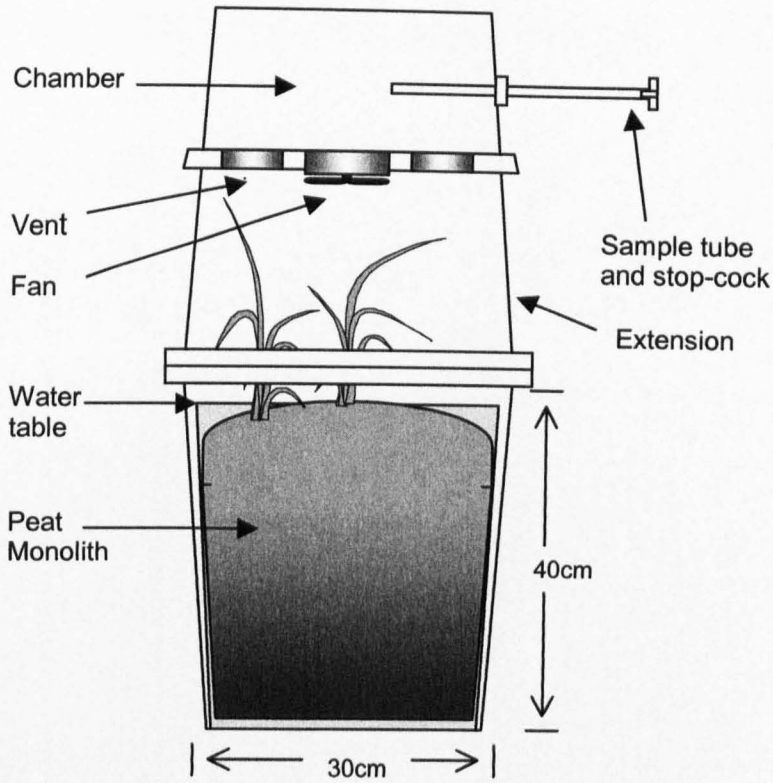

Figure 2.5: Experimental methods for CH_4 flux analysis from monoliths.

duration of the experiment. The monoliths were also watered regularly with de-ionised water in order to maintain the water table at, or just above the peat surface.

The monoliths were transferred to three controlled environment cabinets (CONVIRONS) in mid-February 1999. This facility affords close control of variables affecting methane emissions. The temperature was maintained at 10°C throughout the experiment and each monolith received 350mls of deionised water per week, which effectively maintained the water table at the surface. In addition, relative humidity was maintained at 80% and light levels were maintained at 300 μEm^{-2} (12h d^{-1}). Methane emissions were measured from each monolith four times per week via a static chamber technique. On each sampling day samples were taken from a 0.02-m^3 headspace, which

was defined by temporarily sealing a chamber (with an extension collar to protect the vegetation) onto the monolith for 40 minutes (fig 2.5). The chamber contained a fan, which allowed good mixing of headspace gases during incubation. During the period of incubation, a 60 ml air sample was withdrawn from each chamber at 0,20 and 40 minutes. Samples were analysed for CH_4 content by GC FID (as in section 2.5) and CH_4 fluxes were calculated by the method discussed in section 2.5. After 4 weeks of measurements (to establish base line of monolith flux behaviour as described in section 2.5) each of the 24 monoliths were randomly assigned either one of four Na_2SO_4 treatments, a NaCl treatment or a control. The four Na_2SO_4 treatments ranged from continuous small weekly additions of Na_2SO_4 amounting to annual rates of S deposition of 15, 50 and 100 kg S-SO_4 ha^{-1} to one treatment of 50 kg S-$SO_4$$ha^{-1}$ that was applied as single dose. The NaCl treatment was applied to replicate the total ionic strength from the 50 kg S-SO_4 ha^{-1} treatment but without the input of SO_4^{2-} ions. This experimental addition provided a test of whether or not any effect of the treatments on CH_4 flux was due simply to changes in ionic strength alone i.e. a 'salt effect' rather than by a SO_4^{2-} induced microbial community/function shift.

2.7 Statistical analysis

To evaluate treatment effects on CH_4 fluxes from a time series of measurements, repeated measures methods of statistical analysis are required. These methods of analysis must be employed for the type of data used in this study, as measurements that are repeatedly taken with time may not be independent, i.e. they are auto-correlated with time. In general, when measurements are taken from manipulation experiments, where a variable is investigated from different plots at a field site at a single time, the

main statistical concern is that the plots are not spatially auto-correlated. This problem can be circumvented by spatially randomising experimental plots, as was done in the experiments reported here, however it is impossible to randomise time points as they must follow their natural sequence (Horgan and Sword, 1995).

In the studies reported in Chapters 3 and 5, repeated measures multivariate analysis of variance (MANOVA) was used (MINITAB, release 11, Minitab inc. USA). This method permits the inclusion of each time-point as a separate variable thereby allowing the examination of within-treatment variability (time effect and interactions between time and treatment (time x treatment)) so that the treatment effect may be evaluated.

Statistical methods that are more specific to individual chapters are discussed where relevant.

Chapter Three

Controls on suppression of methane flux from a peat bog subjected to simulated acid rain sulfate deposition.

(A version of this chapter was submitted to Global Biogeochemical Cycles, as: Controls on suppression of methane flux from a peat bog subjected to simulated acid rain sulfate deposition. With Dise N.B and Fowler D, as co-authors)

3.1 Abstract

The effect of SO_4^{2-} deposition through acid rain on CH_4 emissions from peatlands was examined over a two-year period by manipulating the input SO_4^{2-} to a pristine raised peat bog in northern Scotland. Weekly pulses of dissolved Na_2SO_4 were applied to the bog over two years in doses of 25, 50 and 100 kg S $ha^{-1}year^{-1}$ reflecting the range of pollutant S deposition loads experienced in acid rain-impacted regions of the world. CH_4 fluxes were measured at regular intervals using a static chamber / GC FID method. Total emissions of CH_4 were reduced by between 21% and 42% relative to controls, although no significant differences were observed between treatments. Estimated total annual fluxes during the second year of the experiment were 16.6 g m^{-2} from the controls and (in order of increasing SO_4^{2-} dose size) 10.7, 13.2 and 9.8 g m^{-2} from the three SO_4^{2-} treatments respectively. The relative extent of CH_4 flux suppression varied with changes in both peat temperature and peat water-table with the largest suppression during cool periods and episodes of falling

water-table. These findings suggest that low doses of SO_4^{2-} at deposition rates commonly experienced in areas impacted by acid rain, may significantly affect CH_4 emissions from wetlands in affected areas. It is proposed that SO_4^{2-} from acid rain can stimulate sulfate-reducing bacteria, into a population capable of out-competing methanogens for substrates. It is further proposed that this microbially-mediated interaction may have a significant current and future effect on the contribution of northern peatlands to the global methane budget.

3.2 Introduction

Methane, on a molecule for molecule basis, is some 21 times more powerful than CO_2 as a greenhouse gas [*IPCC, 1996*] making it responsible for an estimated 22% of the present greenhouse effect [*Lelieveld et al., 1998*]. Although the concentration of atmospheric methane has been increasing since the onset of the industrial revolution there has, in recent years, been a slow-down in this growth rate [*Dlugokencky et al., 1998; Dlugokencky et al., submitted*]. This implies either an increase in a methane sink or a decrease in a CH_4 source, although reasons for this declining trend remain elusive.

Microbial decomposition in waterlogged soils, as found in natural wetlands and rice paddies, is the largest source of methane to the atmosphere [*Matthews and Fung 1987; Aselmann and Crutzen 1989; Matthews et al., 1991*]. In such systems diffusion of O_2 below the water table is low and so O_2 is rapidly removed by aerobic micro-organisms. This is followed by microbial reduction of a suite of oxidised inorganic compounds such as (in order of decreasing energy yield for microbes) NO_3^-, Mn IV and III, FeIII and SO_4^{2-} [*van Breemen* and *Feitjel* 1990]. Once SO_4^{2-} has been consumed, the terminal and lowest

energy-yielding step in anaerobic microbial decomposition is the consumption of H_2/CO_2 and acetate to produce methane. Consequently, since reduction of SO_4^{2-} by micro-organisms (SO_4^{2-} reducing bacteria – SRB) provides a more efficient means by which competitive substrates can be consumed, methane-producing bacteria (MP) are placed at a competitive disadvantage [*Abram and Nedwell, 1978; Schonheit et al., 1982; Kristjansson et al., 1982*]. Suppression of methanogenesis by stimulation of SO_4^{2-}-reducing populations explains the observation that salt marshes and wetlands overlying SO_4^{2-} rich deposits emit considerably less methane than otherwise comparable freshwater wetlands [*Bartlett et al., 1987, Rejmankova and Post, 1996*]. Experimental work with large (10^3kg SO_4^{2-}-S) fertilisation doses of SO_4^{2-} on rice paddies [*Dernier van der Gon and Neue, 1994*; *Lindau et al., 1994; Lindau et al., 1998*] has also shown a clear suppression of CH_4 emission. The interaction has also been documented in environments with SO_4^{2-} concentrations at freshwater levels [*Lovely and Klug, 1983*]. This suggests the hypothesis that acid rain, of which a principal component is SO_4^{2-}, may affect the emission of methane from impacted wetlands.

In Eastern Europe and Asia there is a trend of increased SO_4^{2-} deposition due to economic growth [*Bhatti et al., 1992; Rodhe et al 1995; Rodhe 1999*]. This trend of enhanced supply of a species that is known to adversely affect methane production in anaerobic environments therefore deserves close examination as it presents us with a potential mechanism that may explain the observed decline in the atmospheric methane growth rate. Few studies have, until recently, investigated this potentially important link.

In peat core incubation experiments, *Fowler et al.* [1995] showed that single doses of SO_4^{2-} representing annual total deposition rates in acid rain impacted areas (40kg SO_4^{2-} S ha^{-1}) reduced CH_4 fluxes from peat by around 40%. They also found that, following an initial

three week period of suppression, emissions recovered to pre-treatment levels, which implies that large, individual inputs of SO_4^{2-} may create a 'boom-bust' cycle in SRB populations as SO_4^{2-} is either lost gaseously from the system or is converted to more biologically recalcitrant forms.

In field studies of underlying processes in two peatlands with contrasting SO_4^{2-} deposition regimes *Watson and Nedwell [1998]* showed that SO_4^{2-} reduction is indeed an important pathway in the degradation of organic matter, suppressing methane production, albeit to a varying degree depending on the season. No relationship between SO_4^{2-} load and methane production could, however, be deduced due to the confounding presence of other factors, such as differences in the degradability of peat (variable C: N ratio) and differences in NPP at the two sites due to climatic differences.

Dise and Verry [in press] alleviated the problems of inter-site heterogeneity encountered by *Watson and Nedwell [1998]* by manipulating SO_4^{2-} deposition to a bog over a 12-week period during a single growing season. Although the S deposition rate amounted to a maximum of 145 kg SO_4^{2-}-S ha^{-1} $year^{-1}$, at the extreme high end of SO_4^{2-} deposition in both Europe and Asia, individual doses were no higher than 2.7kg ha^{-1}, far lower than in single dose and fertilization experiments conducted thus far and more reflective of the mode of pollutant S deposition experienced in nature. They found that CH_4 emissions were reduced by 30-40%, similar to the level of suppression found in single dose experiments. This work suggests that small, continuous inputs of SO_4^{2-} may have the same net suppressive effect as single large SO_4^{2-} doses, as SRB communities may be maintained at competitive levels by the continuous low-level influx of SO_4^{2-}. They were, however, unable to fully examine the effect that changes in temperature and water table may have on treatment effect since the measurement period was confined to the warm summer months when these

variables remained relatively constant. To my knowledge, no study has examined the effect of SO_4^{2-} on CH_4 emission measured over a full year, nor the interactions between this effect and the temperature and hydrology of a wetland.

The objective of this experiment is to investigate the effect of low, continuous SO_4^{2-} deposition on CH_4 emissions from a peatland by manipulating SO_4^{2-} deposition levels over two growing seasons within a range experienced in areas of the world that are impacted by acid rain. In addition, with natural changes in temperature and water table over this period the degree to which any suppression in CH_4 flux is controlled by climate was investigated.

3.3 Materials and Methods

3.3.1 Experimental site description

The experiment was located on an extensive pristine portion (i.e. unaffected by cutting or drainage) of the Moidach More in Morayshire in the northeast of Scotland (57.46 N, 3.62 W) at an altitude of 275 m above sea level. The raised mire consists of peat of more than 0.5m thick extending over 760 ha and averaging 2.1m in depth. The mean annual precipitation at the site is approximately 900 mm [*Meteorological Office 1987*] and the mean annual temperature is 8°C[*Williams et al. 1999*]. The vegetation mainly comprises *Sphagnum* species, which include *S. magillanicum and S. capillifollium* [Ehrh.] Hedw. and *S. revurvum* [P.Beauv.]. The dominant sedge is *Trichophorum cespitosum* [L.] Hartm. (Deer Grass). Other plants include *Erica tetralix* L. and, in areas of the bog affected through cutting and burning, *Calluna vulgaris* [L.]. The site was selected for its low ambient SO_4^{2-}-S deposition rate of 5kg $ha^{-1}year^{-1}$ [*R.Smith pers. comm.*]. Rainfall data for

the area was collected at Grantown on Spey, approximately 10km south of the study site (supplied by the British Atmospheric Data Centre).

3.3.2 Sulfate-S Applications

Wooden boardwalks were installed within the sampling area to allow repeated access while minimizing site disturbance during sampling. 20 experimental plots (2 x 2m) were established on an area of the peatland that exhibited uniform characteristics in terms of its vegetation, topography and hydrology. The plots were separated by a 1m buffer strip and were randomly assigned to one of three treatments or a control. The treatments consisted of additions of 20, 45 and 95 kg SO_4^{2-}-S per hectare per year applied as Na_2SO_4. In addition to annual ambient deposition, this amounted to total annual deposition rates of 25, 50, and 100kg SO_4^{2-}-S ha^{-1} respectively. The treatments were applied as weekly doses of between 1.2 and 4.7 mmol SO_4^{2-}-S in 1 litre of deionized water, which amounted to between 0.5 and 1.9 kg SO_4^{2-} -S ha^{-1} wk^{-1}. Over the winter period (November to March) plots were dosed once a month rather than weekly, and doses were correspondingly four times stronger. SO_4^{2-} was added as Na_2SO_4 (as opposed to H_2SO_4) to minimise any potentially confounding pH effects. Weekly doses of Na_2SO_4 in 1 litre of deionized water were sprayed evenly onto each 2 x 2m plot using a pressurized garden sprayer (Hozelock, UK). The solution added amounted to a hydrologically negligible weekly increase of 0.25mm of water to the system. Controls received the same volume of deionized water. All experimental additions began on 25th June 1997 following five weeks of CH_4 flux monitoring at the site.

3.3.3 Methane flux measurements

CH_4 flux was measured using static chambers, which were semi-permanently (for the duration of the experiment) placed within each experimental plot. The chambers consisted of sections of polypropylene pipe (length 25 cm, internal diameter 30cm). A groove was machine cut into the top edge of each section to accommodate a neoprene 'o' ring. The bottom edge of each section was bevelled to facilitate installation of the chambers to a depth of 2-3cm into the peat surface. Once in position the chambers were not moved again for the duration of the experiment.

The headspace volume was defined by placing a transparent acrylic lid onto the 'o' ring. Each lid was fitted with a silicone rubber septum (Suba Seal) which allowed repeated sampling of the headspace gas with a needle and syringe, both immediately after enclosure and then again 20 minutes after the initial sample was taken. Three way stop-cocks allowed samples to be stored in the syringes prior to analysis (within 24 hours) on a GC FID (Chrompack CP9000) with a 2m long Poropack Q column connected to a Spectra Physics integrator. At bi-monthly intervals, the headspace of the chambers was sampled repeatedly (a minimum of 3 samples) over an hour-long period to test for linearity in the increase of methane concentration. Each plot was sampled weekly (late spring – early autumn) to monthly (late autumn – early spring) starting in May 1997. Samples were taken within an hour of noon on each sampling day. Chambers were sampled for CH_4 flux prior to SO_4^{2-} applications at all times.

3.3.4 Additional Measurements

On each sampling day, peat temperature (0, 5, 10, 15, 20, 30, 40 and 50cm below the peat surface) was measured using a thermocouple probe (ATP Technology, UK) at three locations spanning the experimental area in the bog. Water table was also monitored using 'dip wells' (50 cm lengths of 3cm diameter polycarbonate tubing, which were positioned 0.5-1m from each static chamber. Since vascular plants provide a major conduit of CH_4 release and are in some cases the dominant means by which methane is emitted to the atmosphere [*Schimel 1995*], the density of the dominant vascular plants (*Trichophorum cespitosum*) in the plots was calculated by periodically (once in August 1997, monthly in 1998) counting the number of individual live shoots within each chamber.

3.3.5 Pore Water Chemistry

In the autumn of 1998, pore water was collected from three depths below the peat surface (10, 20 and 30cm) of control and 50kg continuous S treatment plots. Samples were collected using 'sippers' which were positioned in the peat. These were constructed from 20mm external diameter polycarbonate tubing containing an inverted 10ml polypropylene syringe at the base which served as a pore water reservoir. The large, syringe plunger openings were sealed off and 1mm perforations were drilled into the side to allow lateral inflow of surrounding pore waters. The upwards pointing, needle end of the syringe was connected to a three way stopcock valve above the peat surface via a 30 cm length of 1.5mm internal diameter Teflon tubing. This minimised exposure of pore waters in the syringe reservoir to oxic conditions. Sippers were positioned at the three depths in four control and four 50 Kg S continuous treatment plots. Samples of pore water were taken in

November 1998 and were drawn into syringes fitted with three way stop cocks while ensuring the sample filled the whole syringe volume (no headspace) to ensure anaerobic conditions during storage for transportation back to the laboratory.

Pore water [CH_4] was measured by introducing 5mls of each pore water sample into 40ml boiling tubes (35ml ambient air headspace) fitted with Suba Seal silicon rubber septa. The tubes were shaken for 2 minutes to strip dissolved methane into the headspace. Headspace samples were analysed for CH_4 by GC FID (see above) and were corrected for CH_4 concentration in ambient air [*Dise 1993*].

Remaining pore water samples were filtered with 0.45 μm membrane filters (Whatman) under vacuum and frozen prior to analysis by ion chromatography.

3.3.6 Calculations and Statistical Analysis

Methane fluxes are expressed in mg CH_4 m^{-2} day^{-1} by calculating the linear change in CH_4 concentration over time, from within a chamber of known volume enclosing a known area of peat. Temperature 10 cm below the water table (close to the zone of maximum CH_4 production [*Daulat and Clymo 1998*]) was calculated by interpolating temperatures measured at different depths beneath the peat surface. To evaluate the effects of water table and peat temperature on net CH_4 flux from the site (all treatments pooled and averaged) we employed multiple linear regression analysis (MINITAB, release 11, Minitab inc. USA). The total mass of CH_4 emitted from the different treatments was estimated by integrating flux measurements over time. Treatment effects on CH_4 fluxes were determined by repeated measures multivariate analysis of variance (MANOVA) (MINITAB, release 11, Minitab inc. USA) with sampling time points included as a

variable and sedge shoot density included as a covariate. This method permitted the evaluation of within-treatment variability (time effect and interactions between time and treatment (time x treatment)).

3.4 Results

3.4.1 Methane emissions

Methane emissions over the course of the experiment show a distinct seasonal pattern, with peaks in emission during the warm summer months and lower emissions during cooler winter periods (Fig 1). Between 1997 and 1998 distinct interannual differences in emission are evident, with total emissions in 1997 (7.7g CH_4 m^{-2}), 51% lower than over the same measurement period in 1998. These differences correspond to a lower water table in 1997 than in 1998 (fig. 2), which is due to lower-than-average rainfall in 1997 (670mm, 26% lower than the 1916-1950 mean) and slightly lower than average rainfall in 1998 (860mm, 4% lower than the mean). Over the April to September growing season, when most CH4 is emitted, there was 26% less rainfall in 1997 than 1998. The time of maximum emission also differed between the two years, peaking in early July in 1997 (37.4 mg CH_4 m^{-2} day^{-1}) and in late September/early October in 1998 (139.7 mg CH_4 m^{-2} day^{-1}).

Period	Regression		n	r^2
1997	log(flux+1)	$= 0.0092(WT) + 0.0698(T) + 0.642$	19	0.64*
1998	log(flux+1)	$= 0.0388(WT) + 0.0407(T) + 1.406$	26	0.32*
1997+1998	log(flux+1)	$= 0.0273(WT) + 0.0512(T) + 1.178$	45	0.38[a]

*$P < 0.01$, [a] $P = 0.05$, n = the number of sampling dates during each period.
r^2 = explained variance

Table 3.1: Relationship between CH_4 flux ($mg/m^2/day$) (pooled and averaged for all plots per sampling period) and water table (*WT*/cm) and temperature (*T*°C) 10cm below the water table.

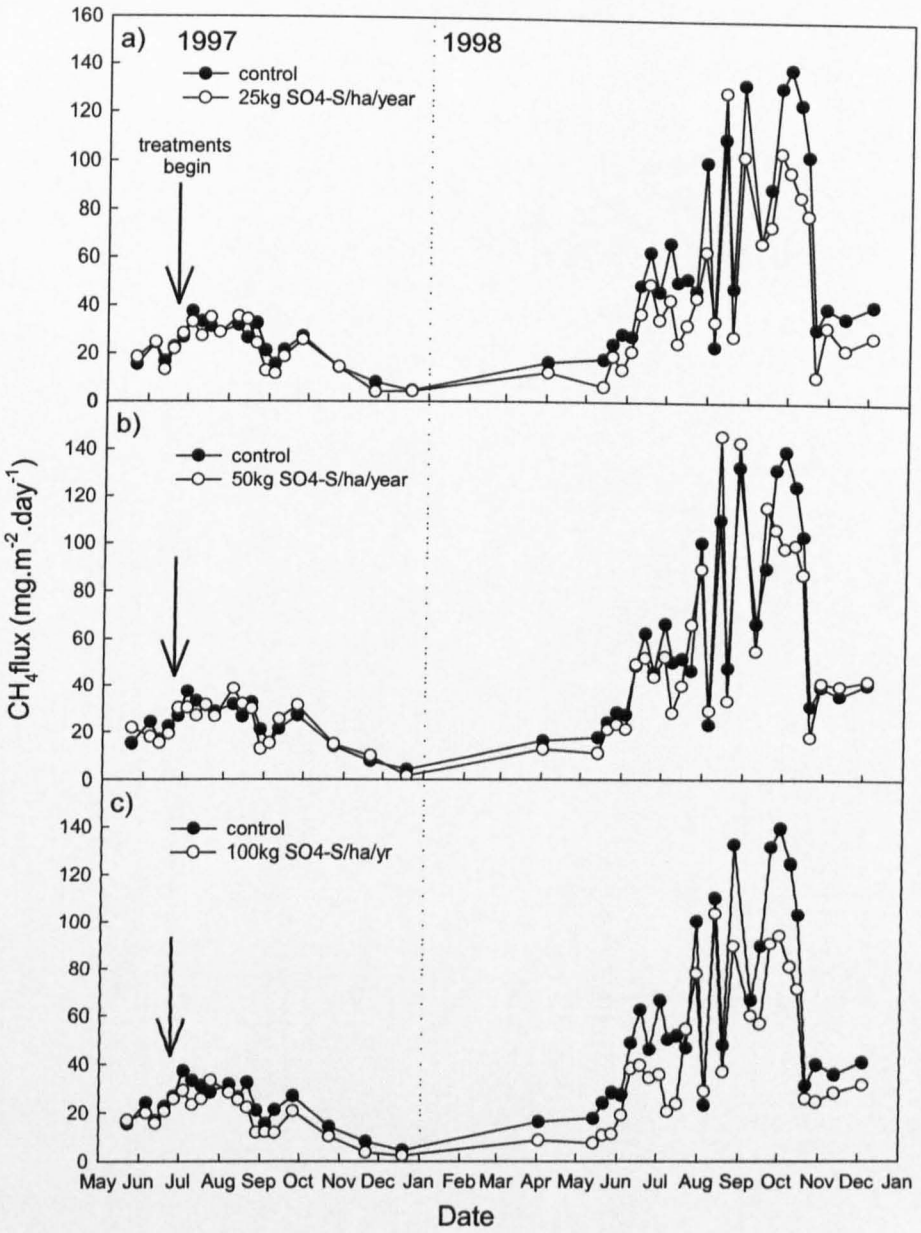

Figure 3.1: Methane emission from plots in Moidach More (1997-1998) treated with different sulfate deposition rates of 25, 50 and 100 $KgSO_4$-S/ha/year (a,b, and c respectively) against emissions from control plots. Each point represents the mean flux of 5 measurements. Error bars are omitted for clarity.

After log-transforming the skewed flux data, multiple linear regression analysis of mean weekly data (all treatments combined) from 1997 showed that both water table (*WT*) and temperature (*T*) explained the majority of variability in log CH_4 flux (table 3.1) however, in 1998 only a third of the variability could be explained by these two variables. This indicates that some other variable (or variables) was driving most of the variability in

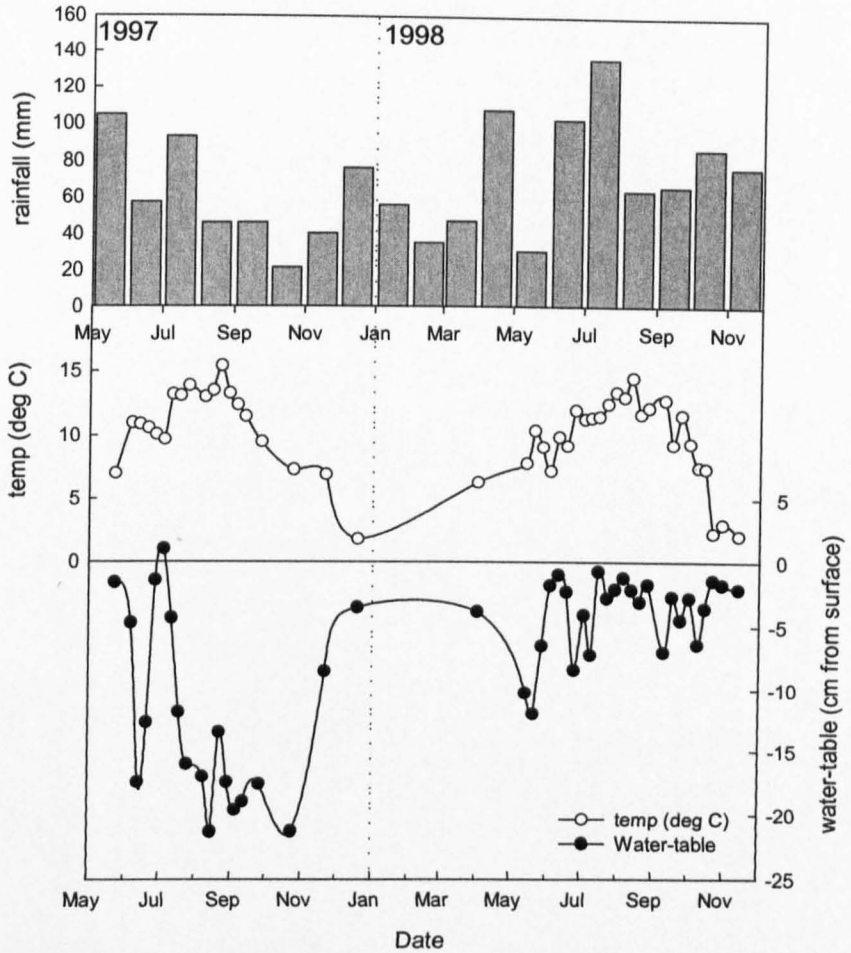

Figure 3.2: Total monthly rainfall (a), peat temperature 10 cm below water table (b) and mean water-table position (c) over the course of the experiment.

1998. Changes in water table were of a smaller magnitude in 1998 than in 1997 (Fig 3.2) and methane emissions during the warm summer months of 1998 exhibited spikes that were unattributable to measured variables.

3.4.2 The role of vascular plants in CH4 emissions

As anticipated, and as previously reported by Schimel [1995], fluxes were well correlated with sedge shoot density on individual sampling dates during the pre-treatment phase of the monitoring program ($r = 0.68$, $P < 0.001$, $n=19$). This relationship accounted for the majority of within-treatment variation on any individual sampling date during that period.

Since competing micro-organisms are known to form intimate relationships with plant roots [*RooneyVarga et al.*, 1997; *Watson and Nedwell* 1998; *Kusel et al.*, 1999; *Wind et al. 1999*] any effect of treatment on subsequent (post treatment initiation) changes in the relationship between fluxes and shoots of *Trichophorum* could not be discounted.

TREATMENT	Mean CH_4 Flux (±s.e.) (mg CH_4 .m^{-2}.day^{-1})		P-value *(Control vs. Treatment)* (a)	(b)
Pre-treatment (21st May – 25th June 1997)	n = 25			
Control	21.2 (3.5)			
25 kg SO_4^{2-}-S designate	21.3 (3.4)		0.98	0.95
50 kg SO_4^{2-}-S designate	21.0 (2.9)		0.96	0.30
100 kg SO_4^{2-}-S designate	19.8 (1.9)		0.74	0.61
Post- treatment year 1. (2nd July – 17th Dec 1997)	n = 70			
Control	23.8 (2.7)			
25 kg SO_4^{2-}S. ha^{-1}.yr^{-1}	22.1 (2.3)		0.62	0.48
50 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	23.4 (2.2)		0.91	0.11
100 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	18.6 (1.7)		0.09	0.07
Post-treatment year 2. (31st March – 11th Nov 1998)	n = 130	*(Total 1998 flux (g/m^2))*		
Control	64.8 (5.4)	*(16.8)*		
25 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	48.8 (3.8)	*(10.7)*	<0.01	<0.001
50 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	58.8 (4.5)	*(13.2)*	0.35	<0.001
100 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	45.2 (3.2)	*(9.8)*	<0.001	<0.001

Table 3.2: Summary table of average methane emissions over three monitoring periods. P-values indicate significance of treatment (treatment vs. control) effect on mean daily flux as evaluated from repeated measures MANOVA (Wilk's Lambda test) both without a covariate (a) and including *Trichophorum* sedge density (shoots/m^2) as a covariate (b). No significant 'time x treatment' interactions were observed. The total flux of CH_4 in 1998 was calculated by integrating the mean daily flux over time.

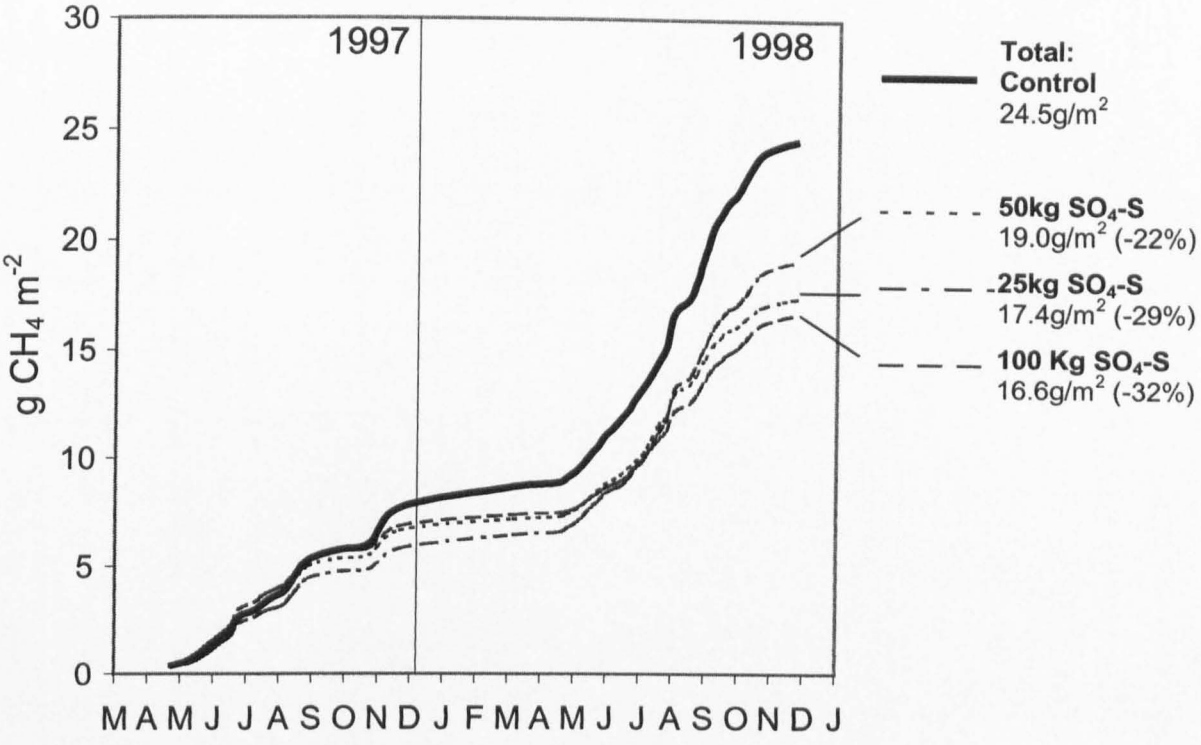

Figure 3.3: Cumulative mean daily methane flux from Moidach More.

3.4.3 SO_4^{2-}-S deposition experiments

Over the course of the first year differences between daily CH_4 fluxes from control vs. treatment plots were not significant (table 2; fig. 1). However on adjusting fluxes to sedge density (which explained most of the variability within treatment groups on individual sampling dates) the highest dose treatment (100 kg-SO_4-S $ha^{-1}yr^{-1}$) was of borderline statistical significance (n=70, P= 0.07) (table 2).

In 1998 both the 25 and 100 kg-SO_4^{2-}-S $ha^{-1}yr^{-1}$ treatments exhibited significantly smaller fluxes than the controls over the entire year (n=130 RM-MANOVA, $P<0.01$ and $P<0.001$ respectively). With mean fluxes over the year amounting to 48.8 and 45.2 mg CH_4 m^{-2} day^{-1}, for the 25 and 100 kg SO_4^{2-}-S $ha^{-1}yr^{-1}$ respectively, against 64.8 mg CH_4 m^{-2} dy^{-1} emitted by the control plots, this amounted to a mean annual suppression of 25% and 30%

(table 2). Mean fluxes from the 50 kg-SO_4^{2-}-S $ha^{-1}yr^{-1}$ treatment were not significantly lower than controls which was probably due, by chance, to their having the highest sedge density out of all treatments and controls (40% higher than controls in 1998). On inclusion of sedge densities as a covariate all treatments showed highly significant suppression in fluxes relative to controls at the p=0.001 level. This analysis by repeated measures MANOVA also showed that there was no time-treatment interaction, nor were significant treatment-to-treatment differences observed. Over the 2-year length of the experiment, treatment plots emitted between 22% and 32% less CH_4 than controls (mean net fluxes versus 24.5 g m^{-2}, respectively) (fig.3). However, when considering only 1998 emissions, time integrated mean daily fluxes show a far stronger suppression of fluxes with respective suppressions of 36%, 21% and 42% (in order of increasing SO_4^{2-} dose rate) from the total control CH_4 flux for 1998 of 16.8g m^{-2}.

By taking the proportional difference in CH_4 flux measured between control and treatment plots before treatments began as our best estimate of background (non-treatment) variability, we are able to estimate the relative extent to which CH_4 flux from the treatment plots is lowered over the course of the experiment. This is done by calculating for every post-treatment flux, the value of ΔCH_4 (percentage change in CH_4 flux), defined as:

$$\Delta CH_4 = [y_1 (x_a / y_b) - x_1] / x_1 \,.\, 100 \qquad (3.1)$$

Where ΔCH_4 is the percentage change in methane flux as the result of a treatment effect; x_1 and y_1 are the respective control and treatment fluxes during the treatment period and x_a and y_b are the respective mean control and treatment fluxes prior to the start of treatment

applications. Therefore the more negative the value of ΔCH_4, the lower the CH_4 flux of treatments relative to controls.

After an apparent initial stimulation in CH_4 flux from treated plots (25 and 100 kg SO_4^{2-}-S) in July and early August, all treatments show a trend of increasing difference between treatments and controls until the spring of 1998 (Fig. 4). Thereafter, the relative difference between treatments and control fluxes varied over time.

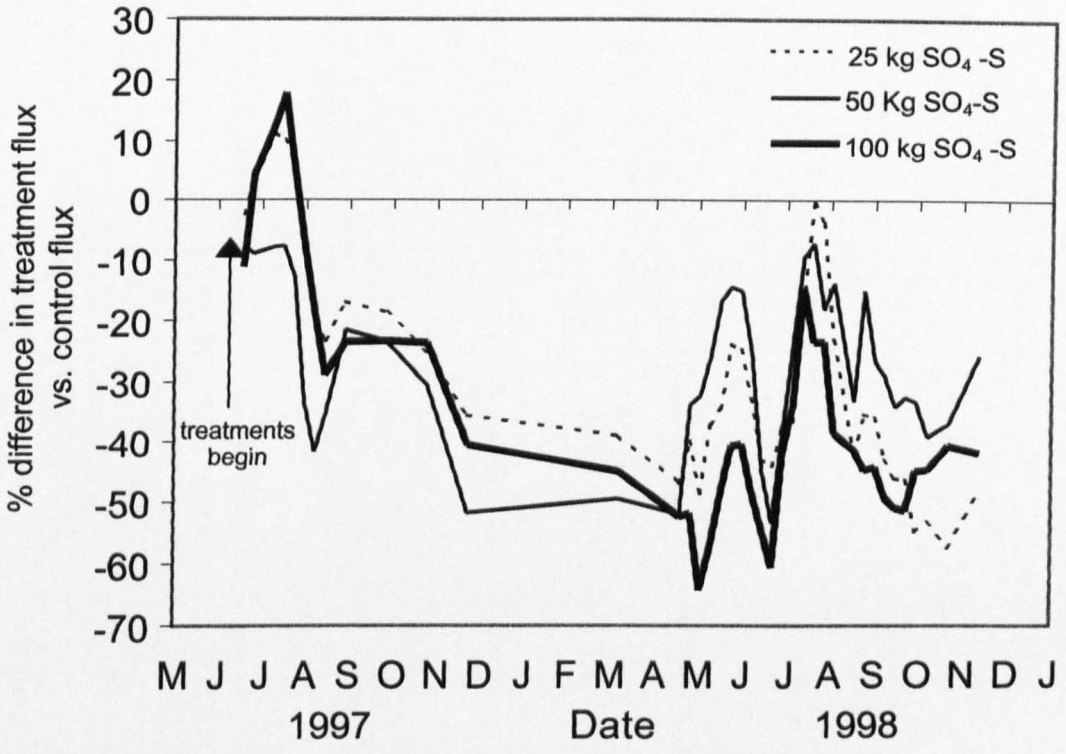

Figure 3.4: Percentage change in treatment CH_4 flux relative to CH_4 flux from controls (Percent variation in CH_4 flux ($PVCH_4$) calculated from equation 1) i.e. negative values indicate a relative suppressive treatment effect and positive values indicate a relative stimulation in treatment CH_4 flux. lines represent a moving average (3 time points) of $PVCH_4$ starting at the onset of the treatment applications.

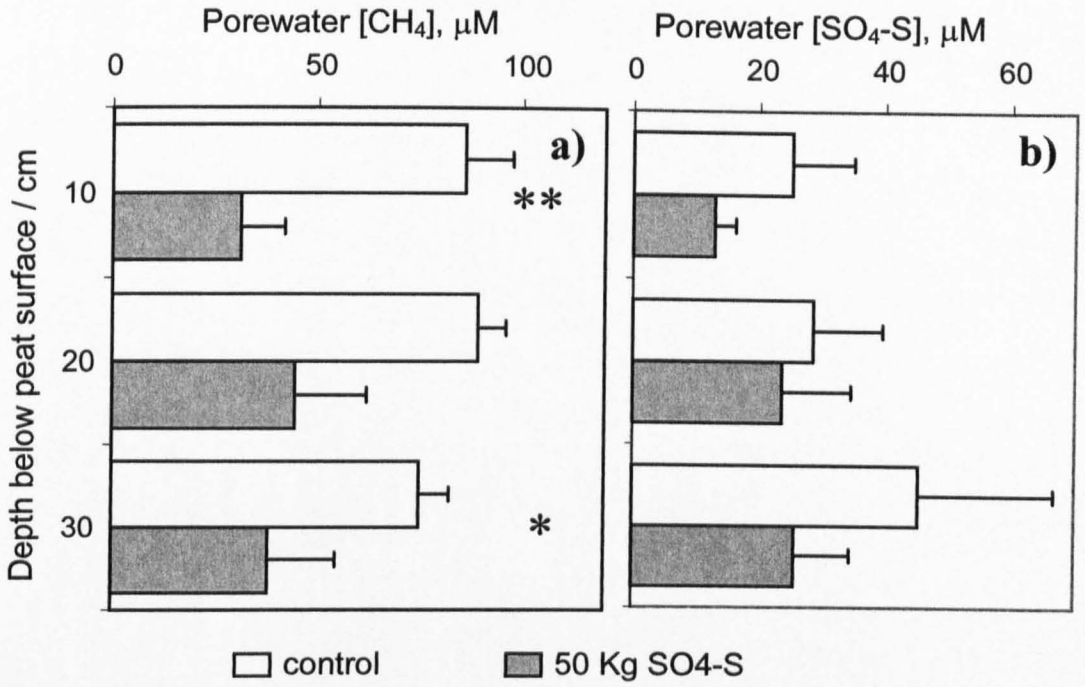

Figure 3.5: Depth profiles of (a) dissolved porewater CH_4 (n=4) and (b) dissolved porewater SO_4^{2-}-S from Moidach More samples taken in November 1998 (n=4). Error bars indicate one standard error of the mean. * indicates significant difference ($P < 0.05$) between treatment and control with two sample t-test. (** $P < 0.01$)

Pore waters collected (10cm, 20cm and 30cm below the surface) from both the controls and the 50kg SO_4^{2-}-S continuous treatment in November 1998 demonstrated that significantly less CH_4 (64%, 50% and 49% lower respectively) was present as a dissolved gas in the treated plots (fig. 5a). Pooling data from all three depths, in the control plots, CH_4 concentrations ranged from 8 to 112 µM and from 1 to 72 µM in the treated plots. Differences were also apparent in SO_4^{2-} concentrations with consistently (although not significantly) smaller concentrations in the treatment plots (fig 5b). When pooling results from the three different depths, SO_4^{2-} concentrations varied between 7 and 107 µM in controls and between 6 and 56 µM in the treated plots. There was a trend of increased concentration of SO_4^{2-} with increasing depth in the peat.

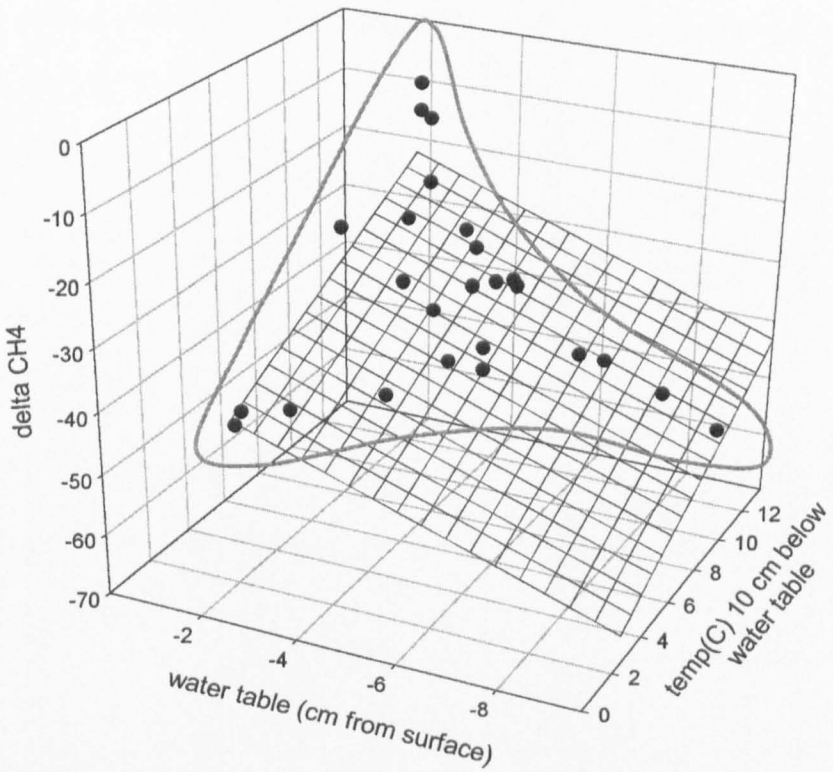

Figure 3.6: Measured 'S treatment effect' (moving average of pooled 25, 50 and 100 kg SO_4^{2-} S data (•)) and modelled data surface showing the relationship between treatment effect and moving averages of both temperature and water table (specific to Moidach More where water-table varied temporally). Heavy lines excludes areas for which no data are available. $R^2 = 0.56$, $P < 0.0001$, $n = 25$

3.4.4 Interaction between water-table, temperature and CH_4 flux in SO_4^{2-} -S treatment plots.

Multiple non-linear regression analysis was used to assess how the response to temperature and water table affected the degree to which CH_4 fluxes were reduced in the SO_4^{2-} treated plots. Only data from 1998 were used in the analysis. The analysis showed that, for all three SO_4^{2-} treatments, the degree of suppression on CH_4 emissions is strongly linked to changes in temperature and water table. ΔCH_4 values from the different treatments were

averaged (3 time point moving average) to give a broad indication of the effect of mean peat temperature and water table position (also 3 time point moving averages) on the extent of CH_4 flux suppression. Multiple non-linear regression analysis yielded a highly significant relationship between ΔCH_4 in 1998 and temperature and water table for all 3 continuous SO_4^{2-} treatments considered separately, as well as for the three treatments lumped together (r^2=0.56, p=0.0001, n=24, fig 3.6) (Table 3.3). This analysis implies that the suppressive effect of SO_4^{2-} on CH_4 flux (i.e. more negative ΔCH_4) increases with both decreasing temperature and declining water table (within ranges of 0-15°C and 0-10 cm below the peat surface).

Treatment $KgSO_4^{2-}$ S/ha/yr	Regression	r^2
25	$\Delta CH_4 = 3.2\,(T) - 2.2\,(WT) - 58.6$	0.59***
50	$\Delta CH_4 = 1.5\,(T) - 2.0\,(WT) - 34.0$	0.29*
100	$\Delta CH_4 = 1.3\,(T) - 3.7\,(WT) - 41.9$	0.61***
Combined 'S-effect'	$\Delta CH_4 = 2.0\,(T) - 2.7\,(WT) - 44.8$	0.56***

*P < 0.05, **P< 0.01, ***P< 0.001; r^2 = explained variance, n = 24 throughout.

Table 3.3: Relationship between ΔCH_4(%) for different treatments (derived from equ. 3.1) water table (*WT*/cm) and temperature (*T*°C) 10cm below the water table .

3.5 Discussion

3.5.1 Seasonal and interannual variability in CH_4 flux

Overall, CH_4 fluxes exhibited typical seasonal changes which broadly followed changes in temperature (figs. 1 and 2b) i.e. higher in the warm summer months and early autumn and low during cool winter periods [*Dise* 1993; *Shannon and White* 1994; *Saarnio et al.*, 1997]. There is a major difference in CH_4 flux between the two sampling years which reflects large differences in water table between the two years, with the field site receiving 26% less rainfall in 1997 than in 1998. With a lower water table there is a decreased volume of potential CH_4 production in the peat column as well as an enlarged oxic layer within which a large proportion of CH_4 is oxidised [*Daulat and Clymo 1998*].

A further consideration is when the rainfall occurred. The July 1997 total of 93mm is misleading as two thirds of the total rainfall fell in the first two days of the month, when the water-table was already high (1.4cm below the surface). It is likely that a large proportion of this precipitation ran off of the peat surface. While the possibility that this level of high-intensity precipitation may have removed, through surface runoff, some of the sulfate from a treatment given 6 days before the high precipitation event, cannot be eliminated it is likely that the 13 mm of rainfall that fell in the area during the intervening time was sufficient to have 'watered in' the applied sulfate, and that the low hydraulic conductivity of peat limited lateral redistribution. Furthermore, it has been shown in a UK mire that SO_4^{2-} is immediately assimilated by the peat system at high water tables (<5cm below the peat surface) and, where water-table is lower than 5cm below the surface, uptake by a combination of assimilatory and dissimilatory sulfate reduction, occurs after a

maximum lag of 4 days [*Brown and Macqueen 1985*]. In this system the water table was 1.4cm below the surface.

Over both years, fluxes show a pattern that is related to temperature, however, in the warm summer and early autumn months of 1998, emissions from all sites varied on consecutive weekly sampling dates by as much as an order of magnitude (fig. 3.1, mean fluxes shown). This phenomenon has been observed at sites that are similarly highly productive in terms of their CH_4 output [*Dise et al 1993; Romanowicz et al., 1995*] and it has been suggested that this may be the result of changes in atmospheric pressure [*Mattson and Likens, 1990*], where low pressure may allow the release, as finite pulses, of large stores of dissolved methane that have accumulated in the peat. It is likely that the weaker relationship exhibited between CH_4 fluxes, water table and temperature in 1998 (Table 1) is due, at least in part, to such pulsing in emissions.

3.5.2 *Effect of SO_4^{2-} treatment on CH_4 emissions*

These experimental data demonstrate that an enhanced, chronic low-level supply of "acid rain" SO_4^{2-} suppresses the emission of CH_4 from wetland soils. The absence of any significant effect during the first year is likely to have resulted from the very low water-table conditions which characterised the summer and autumn of 1997. It is likely that this will have limited the amount of SO_4^{2-} reaching the anaerobic zone during this initial treatment period, thereby limiting the potential for stimulation of microbial competition.

Over the duration of the experiment, between 22% and 32% less CH_4 was emitted from plots treated with SO_4^{2-} relative to control plots (Fig 3). This compares with results of a similar SO_4^{2-} dose experiment where the weekly application rate was 50% higher than the

highest dose rate we applied (maximum of 145 kg SO_4^{2-} -S ha^{-1} yr^{-1}) [*Dise and Verry 2001*]. In addition, the level of suppression of CH_4 flux by the different continuous SO_4^{2-} treatments varied over time (fig 3.4). During the spring of 1998, at the highest level of inhibition, treatments fluxes were suppressed by as much as 50% to 60%. This is within the range of inhibition resulting from single application SO_4^{2-} treatments of several orders of magnitude larger than those applied here in small regular pulses [*Dernier van der Gon and Neue, 1994*; *Lindau et al., 1994; Lindau et al., 1998*].

One interesting and unexpected outcome from these experiments is that there are no significant differences between fluxes from treatments of different amounts of SO_4^{2-}. It has been shown that at low (or below detection limit) SO_4^{2-} concentrations, sulfate reducing bacteria (SRB) may be sustained by fermentatively degrading higher chain fatty acids (e.g. propionate [*Krylova et al 1997*]) and that levels of resulting hydrogen are regulated through consumption through "inter-species hydrogen transfer" by methanogens [*Conrad et al., 1987, Tatton et al., 1989*]. The addition of SO_4^{2-} may stimulate a change in this mutually beneficial arrangement by switching SRB to more energetically beneficial SO_4^{2-} reduction [*Raskin et al.,1996; Schink 1997*]. This has the dual effect of both depriving H_2 utilising methanogens of a major substrate source and allowing SRB to become competitively superior to methanogens [*Raskin et al., 1996*]. Through the addition SO_4^{2-} and an SRB inhibitor (molybdate) *Watson and Nedwell* [*1998*] found evidence to suggest that this "syntrophic" association exists in peatland microbial communities.

In this study, the lowest SO_4^{2-} application rate may well exceed the threshold for SRB to switch from methanogens to SO_4^{2-} as electron acceptors, whereupon beyond such a threshold no further suppressive effect will be evident as other factors such as substrate availability become limiting.

The finding that pore-water CH_4 concentrations were lower in plots treated with 50 kg SO_4^{2-} ha^{-1} yr^{-1} than in controls (fig. 3.5a) indicates that suppression of CH_4 is indeed occurring at source rather than by the means of CH_4 transport to the atmosphere being affected in any way. *Shannon and White* [1996] found that elevated pore-water sulfate corresponds with a zone of depleted CH_4. However, these data show that SO_4^{2-} concentrations tend to be lower (although differences are not significant) in plots treated with 50 kg SO_4^{2-} ha^{-1} yr^{-1} (fig. 3.5b). One can speculate that SRB in these plots have been "activated" to sulfate reduction by the enhanced sulfate supply and can now out-compete methanogens. In doing so the population of SRB may have increased such that they are able to reduce SO_4^{2-} concentrations to a level that is lower in treated plots than is found in controls.

3.5.3 *Variability in extent of suppressive treatment effect*

Not only is there an overall suppression of CH_4 flux from Na_2SO_4 treatment plots but the level of suppression is significantly related to changes in water-table and peat temperature (table 3.3, fig. 3.6). In May and early June 1998, the suppressive effects of the treatments decreased with increases in temperature, as described in the regression equation effect of temperature (table 3.3, fig. 3.4). However, in late June and July, while temperatures remained high, fluxes from treatment plots were reduced to a level 45% to 60% lower than fluxes from controls. Since this enhanced suppression accompanies a lowering in water table (fig. 3.2), it is also described in the regression equation (effect of water table; table 3.3, fig. 3.6). While there is no pore-water data from this period, it has already been shown that later on in the year CH_4 concentrations were significantly smaller in treated plots than in controls. This suggests that, with lower CH_4 concentrations dissolved in treated pore-

waters, upon a lowering of the water table, there is less accumulated CH_4 available to be released to the atmosphere. An additional effect of water table lowering may be the reoxidation of reduced sulfur compounds in unsaturated surface peat, which in SO_4^{2-} treated plots, may provide a temporarily more enriched supply of sulfate to microbial communities than would occur in controls [*Freeman et al., 1994*].

The observation that the difference between treatment and control fluxes is greatest when peat temperatures are lowest was also found by *Nedwell and Watson* [1995], who reported that proportionately less carbon flowed via SO_4^{2-} reduction during warm summer months than via methanogenesis. They inferred that this was due to SO_4^{2-} becoming limiting, as the dissolved pore-water SO_4^{2-} pool decreased during the summer months. I have shown that the degree of CH_4 suppression is still less in summer than during cooler periods even when maintaining relatively high SO_4^{2-} inputs during this time (up to 8.3 kg SO_4^{2-} ha^{-1} $month^{-1}$, close to the total amount of SO_4^{2-} deposited annually on the peatland examined by *Nedwell and Watson [1995]*).

It is possible that the lower suppression in summer may have more to do with seasonal changes in substrate supply and methanogenic pathway than the availability of SO_4^{2}. Several studies have shown that the main methanogenic pathway in peatlands shifts from CO_2 reduction (H_2 substrate) during cool periods to acetate fermentation during the warmer growing season [e.g. *Kelley et al., 1992; Avery et al., 1999*]. This has implications for competitive interactions between methanogens and SRB (when sufficient SO_4^{2-} is available) as uptake rates for H_2 and acetate vary between the two populations. One hypothesis is that SRB have a higher H_2 uptake rate than do methanogens [*Kristjansson et al,. 1982*] and so during periods of mainly CO_2 reduction (cooler periods) SRB are favoured. Acetate uptake rates, however, while higher in SRB, are far closer to that of

methanogens [*Schonheit et al., 1982*] and so other factors such as temperature may affect competition with SO_4^{2-} reduction favoured at lower temperatures and methanogenesis favoured during warm periods [*Bodegom and Stams, 1999*].

The extent of flux suppression from the treatment plots in 1998, when water-table was high, are close to levels of flux suppression reported from single, large dose experiments on high water-table peat cores [*Fowler et al., 1995*]. Furthermore, suppression of CH_4 flux in the continuous SO_4^{2-} addition experiments continued (to varying extents) throughout 1998 which implies that small pulses of SO_4^{2-} are sufficient to maintain a stimulated and possibly enlarged "SO_4^{2-} reducing" population of SRB. This is in contrast to findings by *Fowler et al., [1995]* which demonstrated a pronounced recovery (after initial suppression) of CH_4 fluxes from cores treated with an individual, large dose of SO_4^{2-}. It is also likely that reoxidation of reduced S compounds to SO_4^{2-} in the rhizosphere will have facilitated the continued availability of SO_4^{2-} for further SO_4^{2-} reduction to take place thereby enhancing the net effect of such small SO_4^{2-} pulses on CH_4 emissions [*Freney et al., 1982*].

3.5 Conclusions

Results from this experiment clearly demonstrate that low rates of SO_4^{2-} deposition, at levels commonly experienced in areas impacted by acid rain, significantly suppresses the annual emission of CH_4 from northern peatlands. Lower concentrations of SO_4^{2-} in peat pore waters from SO_4^{2-} treated plots suggests that ambient concentrations of SO_4^{2-} (< 20 kg SO4-S $ha^{-1}yr^{-1}$) are insufficient to cause a switch in SRB function from syntrophic association with methanogens, into a population capable of actively competing with methanogens over available substrates. It is demonstrated that the flux reduction is strongest during cooler time periods as well as during periods where the water-table is

falling, and is weakest during warm periods if water table is near the surface. It is hypothesised that this variation may be governed by a change in the dominant methanogenic pathway of the peatland system [*Kelley et al., 1992; Avery et al., 1999*] from CO_2 reduction, which strongly favours SO_4^{2-} reducing bacteria, to summer time acetate consumption which, during warm periods, favours SO_4^{2-} -reducing bacteria somewhat less [*Bodegom and Stams 1999*].

With North Atlantic regions having already experienced increased SO_4^{2-} deposition and subsequent decline, and with low latitude regions (Asia in particular) experiencing a trend of increasing SO_4^{2-} deposition, the potential for a perturbation in the wetland CH_4 source strength through such a mechanism presents us with a possible contributory factor behind recent observed variability in the atmospheric CH_4 growth rate.

Chapter Four

Sulfate reduction potential, methane oxidation and methane production in a peat bog subjected to simulated acid rain sulfate deposition

4.1 Introduction

Sulfate reduction, a process performed by a variety of bacteria known collectively as sulfate-reducing bacteria (SRB), has been measured in a wide variety of anaerobic environments including both sulfate-rich and sulfate-depleted waters and sediments (for reviews see Ward and Winfrey 1985; Widdel 1988). There has, however, been relatively little research on the contribution of SRB to the biogeochemistry of peatland soils impacted by acid rain, of which sulfate is a major component, and only a few studies have investigated how changes in the rates of sulfate reduction may affect the production of CH_4 in these systems (Wieder et al., 1990; Nedwell and Watson 1995; Watson and Nedwell 1998).

Isolation of SRB in these low pH environments remains elusive and so peatland populations of SRB have proved difficult to quantify (Chapman *in press*). Despite this

limitation, workers have used the radioactive $^{35}SO_4^{2-}$ tracer method to estimate rates of sulfate reduction (Johnsen and Nishita, 1952) and, in so doing, have improved understanding of the importance of SRB populations in determining carbon flow in peat soils (Wieder et al., 1990; Nedwell and Watson 1995; Watson and Nedwell 1998) although comparisons made between sites receiving different SO_4^{2-} inputs tends to be problematic due to the presence of confounding additional factors (Watson and Nedwell, 1998). In this Chapter, sulfate reduction potential is estimated, over seasonal intervals for peat from a site that has both experienced historically low rates of ambient SO_4^{2-} deposition (control) as well as from experimental plots that were manipulated with enhanced SO_4^{2-} deposition rates (a treatment of 50kg SO_4^{2-}-S ha^{-1} yr^{-1}, Chapter 3). In addition, the potential production of CH_4 from impacted peat soils with different SO_4^{2-} inputs as well as the aerobic CH_4 oxidising potential are investigated. The hypothesis is that SO_4^{2-} will increase SO_4^{2-} reduction potential, decrease CH_4 production which will in turn result in reduced CH_4 oxidation capacity. The intention of the study is to elucidate the dynamics of important microbial populations in peatlands as affected by enhanced SO_4^{2-} deposition from acid rain.

4.2 Methods

Peat cores were collected using a stainless steel corer (50cm length and 5x5cm cross section) from control and 50 kg (continuous low dose) SO_4^{2-}-S plots at Moidach More (see Chapters 2 and 3 for full description of the site and experimental manipulation) and at three time intervals: November 1997, August 1998 and November 1998. Experimental low dose SO_4^{2-} additions began in July 1997 and so sampling took place

after 5, 14 and 18 months, respectively, of enhanced S-deposition. A minimum peat core length of 30 cm was recovered from each plot and three replicate cores were recovered from each treatment. The cores were immediately transferred into plastic sample bags (left open) and placed into a portable anaerobic chamber for transport to the laboratory and storage (2°C for 24 - 48 hrs) prior to analysis. Analyses were carried out at two depths (10 and 20cm below the surface) on each core. Subsamples at both depths were oven dried (105°C for 24 hrs) to calculate dry weights. Pore water SO_4^{2-} concentrations were determined by centrifuging subsamples (3600rpm for 30 min.), filtering the centrifugate (Whatman No. 37) and analysing the filtrate by ion chromatography (Dionex).

4.2.1 Sulfate reduction potentials (SRP)

SO_4^{2-} reduction potential (SRP) was estimated using carrier free $^{35}SO_4^{2-}$ in a radiotracer method (Johnsen and Nishita, 1952). In an anaerobic glove chamber (Don Whitley Mark I) 2.5g sub samples of peat were taken in triplicate from each core at each depth and placed in sterilized McCartney bottles. 250µl of radiotracer was added to each sample via a micro syringe. One of each triplicate set of samples was immediately flooded with 2.5ml of zinc acetate-sodium acetate solution (5% w/v) and then frozen to fix sulfides and to terminate microbial activity for storage. The two remaining samples were incubated in the anaerobic chamber for 3 and 24-25 hrs respectively at a temperature of 20°C prior to flooding with zinc acetate solution and freezing. Frozen samples were digested (Chromium reduction) at a later date (within one month, more usually within one week) with a standard Johnsen and Nishita (1952) apparatus and a

solution of 8ml of freshly reduced $CrCl_3$ solution ($\rightarrow$Cr(II)) (Zhabina and Volkov, 1978) and 4ml of concentrated HCl over gentle heat for 40 min. Volatilised H_2S was trapped in zinc acetate-sodium acetate solution. Subsamples (5ml) from the traps were transferred to scintillation vials to which 5ml of Instagel scintillant (Packard Instruments Ltd, UK) was added for measurement of radioactivity by scintillation counter. Counts were corrected for decay and quenching as described by Chapman and Davidson (in press). SRP was calculated as the slope of the regression of SO_4^{2-} that had been reduced during the incubation against time.

4.2.2 Aerobic methane oxidation potential (MOP)

To measure the potential of the peat to oxidise methane (MOP), 20 g of peat from each core depth was slurried with 80 ml of deionized water for 2 min from which 10mls was pipetted into 50 ml flasks and sealed with rubber stopper. A 1000 Vppm methane standard (300µl) was added via a gas-tight syringe to each flask headspace. Flasks were then shaken on an orbital shaker (25°C, 120 rpm). Headspace samples (250µl) were taken at 3-4 hr intervals over 6-8 hrs for analysis of methane concentration by gas chromatography (FID). The slope of the regression line (CH_4 concentration vs. time) was used to calculate the potential CH_4 oxidation rate (ng CH_4 $g^{-1}hr^{-1}$).

4.2.3 Methane production potential (MPP)

To measure methane production potential (MPP) a 2 g sample of peat was placed in a sealed 50ml conical flask. Flasks were evacuated with a vacuum pump and then purged with oxygen free nitrogen (OFN), a cycle that was repeated three times to ensure anaerobicity. Flasks were incubated at 25°C for 48 hrs and headspace concentrations were measured at 5, 24 and 48 hrs, as described in the MOP procedure above.

Temperatures for all incubations (SRP, MOP and MPP) were far higher than can be expected in natural field conditions. It is therefore important that the rates reported from these incubations are considered as potential rather than actual rates.

Table 4.1: Summary of experimental findings

	Relative difference in CH_4 emission (%) (treatment vs. control)[1]	SO_4^{2-} reduction potential (nmol SO_4^{2-} /g/hr)[a]		Environmental conditions (average 2 months preceding sampling)			CH_4 oxidation potential ($ngCH_4/g/hr$)[b]	
				wt (cm)	*T* °C			
		10[c]	20[c]		10[c]	20[c]	10[c]	20[c]
November 1997								
control		9.1 (3.0)	6.6 (1.9)	-15.8	7.2	7.6	12.1 (0.6)	8.8 (3.9)
50 kg SO_4^{2-} S/ha/yr	-39.9	8.7 (2.8)	5.2 (1.5)				10.5 (6.6)	10.1 (0.4)
August 1998								
control		5.8 (3.0) NS	7.5 (2.1)	-3.7	12.1	11.6	10.2 (3.6)	8.9 (3.2)
50 kg SO_4^{2-} S/ha/yr	-13.4	5.5 (3.3) NS	5.0 (1.4)				9.7 (4.1)	7.5 (0.2)
November 1998								
control		4.8 (0.8)	6.9 (3.1) NS	-3.2	3.6	5.2	13.3 (5.0)	13.1 (4.4)
50 kg SO_4^{2-} S/ha/yr	-33.9	11.5 (4.5)*	6.7 (2.1)				6.5 (4.0)	6.0 (3.7)

[1]Derived from equation 3.1 (see Chapter 3)
[a]Value ± SE, $n = 9$; [b]value ± SE, $n = 3$
*= $P < 0.05$ (when comparing treatment to control by multiple linear regression analysis for sulfate reduction potential)
[c] Depth below peat surface/cm
NS indicates where regressions were not significant ($P>0.05$)

4.3 Results and Discussion

Unfortunately, measurements of CH_4 production potentials were inconsistent; while more than half of replicate flasks showed significant linear increases in headspace CH_4 concentration for samples collected in November of 1997 and 1998, the remainder (including all those sampled in August 1998) exhibited non-linear characteristics with time. Frequently, CH_4 concentrations were large during the first time interval before showing a flat, or negative change in concentrations as time progressed. This possibly indicates that a significant proportion of CH_4 may have remained in solution within the (intact) peat sample matrix during cycles of flask evacuation and purging with OFN, only to subsequently come out of solution during early stages of incubation. It is also possible that sufficient anaerobicity for CH_4 production was not achieved in some of the flasks used, which would explain occasional decreases (i.e. CH_4 oxidation) in headspace

CH_4 concentrations with time. Since these samples are likely to have been compromised by such extraneous factors, they are not included in the analysis. Instead the relative difference in CH_4 emission between control and treatment plots are included as an estimate of the relative inhibition of SO_4^{2-} on CH_4 emission at the time of sample collection (Chapter 3). SO_4^{2-} reducing potentials and CH_4 oxidation potentials were unaffected by such limitations, and are compared to these relative changes in surface CH_4 emissions (Chapter 3).

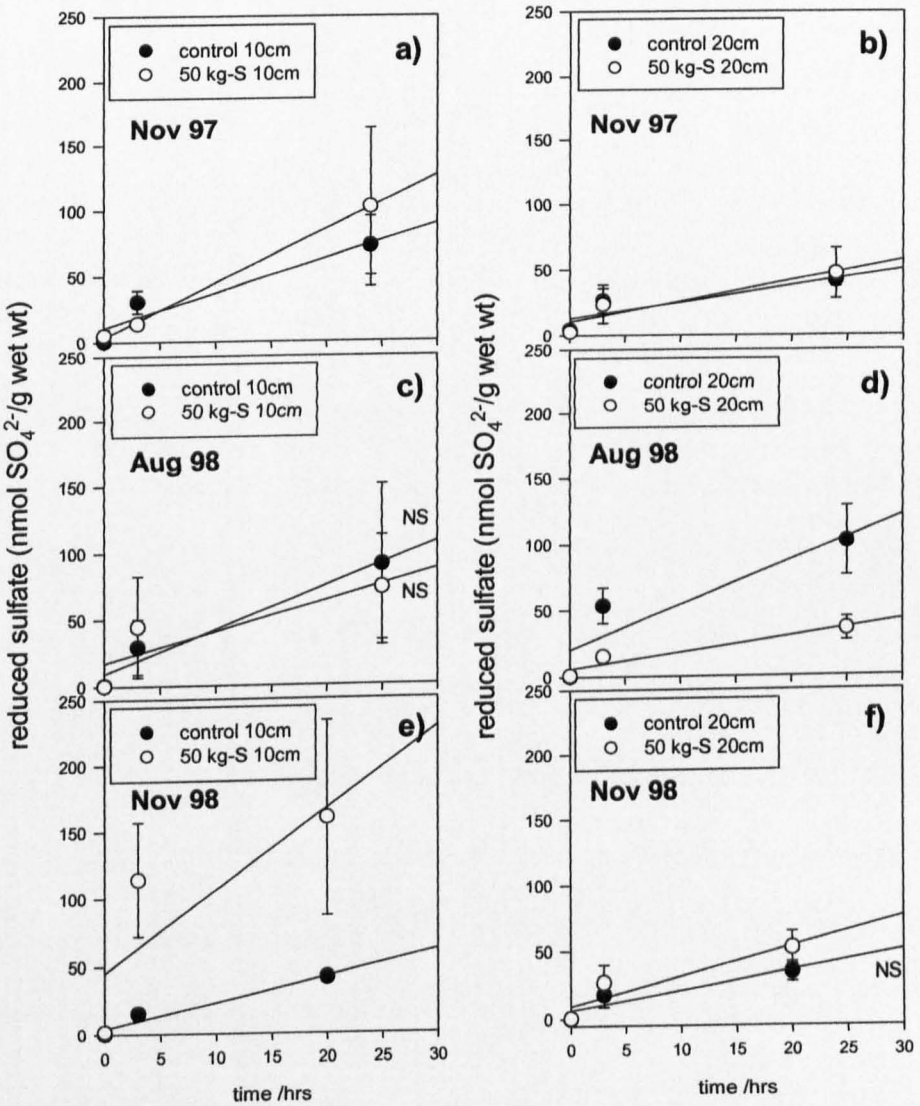

Figure 4.1: Amount of sulfate reduced with time for 3 time periods at 10cm below the peat surface (Nov 97, Aug 98 and Nov 98; a, c and e respectively) and for the same time periods at 20cm below the peat surface (b, d and f respectively). Error bars indicate ± standard error of the mean (n=3). NS indicate where regressions are not significant (P>0.05, n=9).

In November 1998, sulfate reduction potentials were significantly larger ($P<0.05$) 10cm below the surface in the SO_4^{2-} treated plots than in the controls (Table 4.1, fig, 4.1e) even though there was no significant difference in SO_4^{2-} concentration (fig. 4.2). The regression of reduced SO_4^{2-} with time over 20 hours may, however, underestimate potential sulfate reduction rates since the amount of reduced SO_4^{2-} measured after 3 hours was not significantly different to that measured after 20 hours ($P = 0.64$, ANOVA).

With the amount of SO_4^{2-} that had been reduced after only 3 hours from treated peat far exceeding that measured after 3 hours in all other time periods and sample depths (around 120 nmol SO_4^{2-}/g wet peat, fig. 4.1e) and even exceeding reduced SO_4^{2-} measured from other peat samples after 20-25 hours (fig 4.1 a,b,c,d,f), it is most likely that SO_4^{2-} reducing potentials, and therefore inferred SRB population size, were greatly enhanced by the addition of SO_4^{2-} at "acid rain" rates over 18 months. Such an enlarged SRB population will have consumed the $^{35}SO_4^{2-}$ tracer far more rapidly, with the majority of the applied tracer consumed within the first 3 hours (fig 4.1e). Indeed, when considering the difference in reduced SO_4^{2-} with time after 3 hours, the SO_4^{2-} reducing potential was approximately 10 times that of the control for the same time period. This suggests that a far larger SRB population exists in the plots treated with S-deposition than would normally be found in the control plots. This, together with the observation that CH_4 emissions were significantly smaller in treatment plots than in controls supports the hypothesis that acid deposition rate SO_4^{2-} deposition stimulates a competitively superior SRB population, which results in reduction in CH_4 emissions.

Potential sulfate reduction rates during the cool winter period of late November 1997 were generally similar in samples taken from peat 10 and 20cm below the peat surface

(Table 4.1; fig. 4.1a and b) despite a trend of larger concentrations of sulfate at 10cm below the peat surface in those plots treated with additional SO_4^{2-} (fig 4.2). These samples were also taken from a layer of peat that, over the previous two months and due to unusually dry conditions (Chapter 3), was an average of 5 cm above the water-table (Table 4.1) whose position has been shown to define the oxic-anoxic interface in the peat soil column (Benstead and Lloyd, 1994). While some species of SRB are known to be oxygen tolerant (e.g. *Desulfovibrio,* Sigalevich *et al.,* 2000) they are generally obligate anaerobes requiring anoxic conditions for growth and sulfate reduction to take place (Widdel, 1988).

It is unlikely that active sulfate reduction, at similar potential rates to those found below the water-table could be explained by pockets of SRB existing in anaerobic micro-sites since Nedwell and Watson (1995) found only negligible concentrations of reduced S compounds (i.e. FeS, pyrite S and S^o, which would indicate the existence of active sulfate reduction) above the water-table in peat monoliths collected from Ellergower Moss.

It is plausible that the methods utilised in this study, which are similar to those used in other studies (Wieder et al., 1990; Nedwell and Watson, 1995), may stimulate existing, inactive SRB from aerobic peat to sulfate reduction under anaerobic conditions. It has been found that increases in SO_4^{2-} supply may alter the function of existing SRB operating in methanogenic syntrophic consortia into active sulfate reducing population (although only low concentrations of carrier free $^{35}SO_4^{2-}$ were applied as a tracer in this study) (Conrad *et al.,* 1987; Raskin *et al.,* 1996; Watson and Nedwell, 1998). It is thus important that sulfate-reducing potential values reported here are considered more as an indication of relative SRB population size than a measure of their function or the in situ

sulfate reducing activity of the peat. When considering the data in this manner the likely inference is that SRB population sizes remained similar in the control and SO_4^{2-} treated plots at this sample time.

Although there was no evidence that SRB populations differed in November 1997, four months after regular SO_4^{2-} additions commenced, mean CH_4 emission was around 40% smaller in SO_4^{2-} treated plots (Table 4.1, Chapter 3). A hypothesis which may explain these apparently contradictory findings is that there was insufficient time for SRB populations to grow during the first 4 months of experimentally enhanced SO_4^{2-} deposition and so the inhibitory effect of SO_4^{2-} was the result of a functional change in existing SRB lower down the peat profile, that were previously sustained by fulfilling a non sulfate-reducing role (Conrad *et al.*, 1987; Raskin *et al.*, 1996; Watson and Nedwell, 1998).

The low water table during the previous two months may also explain the larger SO_4^{2-} concentration in the upper peat layer, as experimentally applied SO_4^{2-} is likely to have remained in this aerobic layer for some time before reaching the water table whereupon dissimilatory SO_4^{2-} reduction could take place. In addition, reduced sulfur compounds from lower, anaerobic layers will have been reoxidised in the upper, aerobic layers as found by Freeman *et al.*, (1994).

In peat sampled during the summer (August of 1998), a period of high water-table and relatively high temperatures (table 4.1), regressions of reduced sulfate with time yielded no significant relationship at a depth of 10cm for both treatment and controls (Table 4.1), which is most likely due to high variability between replicate samples as indicated by the relatively large error bars (fig. 4.1c). Trend lines, however, indicate that potential SO_4^{2-} reduction rates were statistically the same as in November of 1997.

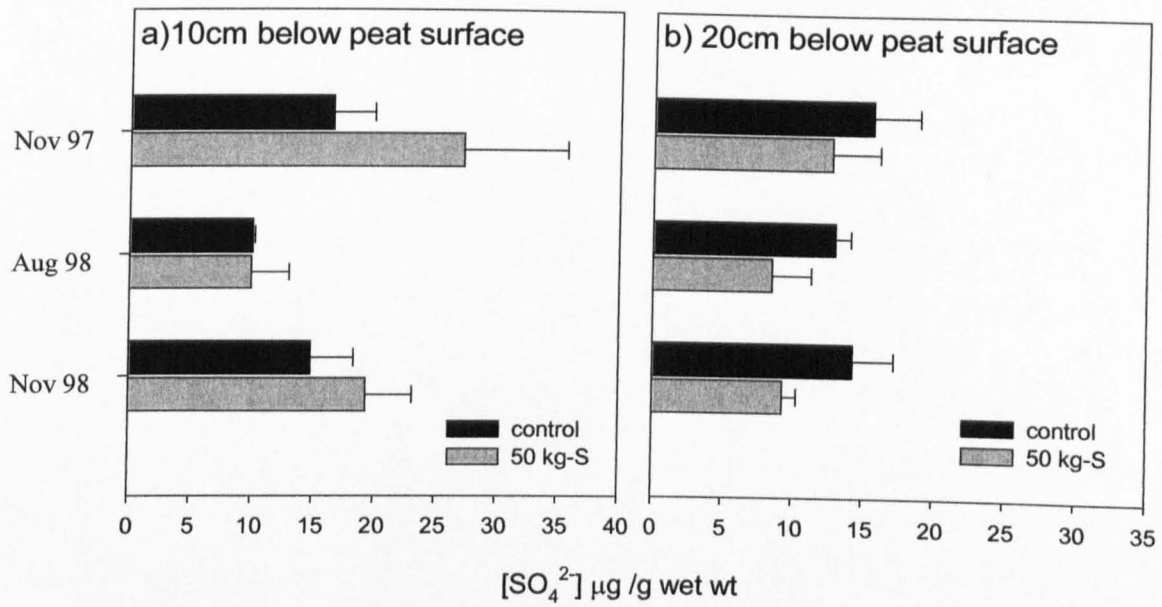

Figure 4.2: Comparison of changes in SO_4^{2-} content of peat over time for control and treated plots at Moidach More, a) at 10cm below peat surface and b) 20cm below peat surface. Error bars indicate ± standard error (n=3).

Concentrations of SO_4^{2-} in August 1998 were smaller at 10cm below the peat surface than during the cooler sampling periods (Fig. 4.2). Shannon and White (1996) and Nedwell and Watson (1995) also found that SO_4^{2-} concentrations diminished during the growing season. With additional inputs of SO_4^{2-} to the treatment plots continuing at weekly intervals during the summer, it is likely that the general decrease in SO_4^{2-} concentrations resulted from temperature-induced enhanced S turnover. In addition plants have been shown to take up a significant proportion of applied SO_4^{2-} as they grow (Chapter 5) so plant growth may also account for this temporal decrease in SO_4^{2-} concentration. Although the reasons for the smaller SO_4^{2-} concentrations during the summer are not fully understood, they do indicate that processes independent of external SO_4^{2-} supply may be responsible as the same pattern was observed in both control plots and plots receiving 10 times the ambient atmospheric input of SO_4^{2-} (i.e. 50 kg-S ha^{-1} $year^{-1}$). Peat sampled from 20cm below the surface showed no significant

variation in SO_4^{2-} concentrations over the seasons. This may suggest that S cycling at depth in the peat profile may be less affected by seasonal changes such as the effect of SO_4^{2-} uptake by plant growth (Chapter 5).

SO_4^{2-} concentrations were smaller in peat samples taken from plots treated with enhanced deposition of SO_4^{2-}. Although differences were not statistically significant, this observation was repeated in peat sampled in November 1998 at a time when CH_4 emissions were significantly smaller (34%) in treated plots than in controls (Table 4.1).

The finding that there is a trend of smaller SO_4^{2-} concentrations in the SO_4^{2-} treated peat than in controls at 20cm during August and November of 1998 was unexpected (fig 4.2 b). Similar findings are presented in chapters 3 and 5, where it is speculated that SRB already existing in syntrophic association with methanogens are activated to SO_4^{2-} reduction by a SO_4^{2-} pulse, and as such, are capable of scavenging ambient SO_4^{2-} to concentrations that are smaller than would otherwise be the case. A shift in the function of existing SRB, without an increase in relative SRB population size, may explain the lack of difference in SO_4^{2-} reduction potential at this depth.

An interesting speculation is that growth of SRB populations in deeper peat may be hampered by substrate limitations that may not factor at shallower depths. SRB populations nearer the surface are likely to have better access to freshly produced labile organic matter. It is plausible that SO_4^{2-} supply to the upper layers of peat, via acid rain, may be sufficient to maintain similar concentrations of SO_4^{2-} in impacted peat and pristine peat, even if SO_4^{2-} reducing potential is larger.

CH_4 oxidation potentials are not significantly different in peat from treatment and control plots (fig. 4.3). When considering changes in CH_4 concentration between the first two time points (when a linear change in concentration is better approximated) a

trend of decreasing CH_4 oxidation potentials at both depths in SO_4^{2-} treated plots relative to controls becomes apparent (Table 4.1). In unpublished experiments, Chapman (pers. comm.) found that small additions of Na_2SO_4 solution (the salt that was applied in this study) had no effect on CH_4 oxidation rates in peat slurries. It is likely that the trend of reduced CH_4 oxidation potential in SO_4^{2-} treated peat reflects SO_4^{2-}-induced reduction in CH_4 production potential in peat. With less CH_4 produced, some methanotrophs may be deprived of CH_4.

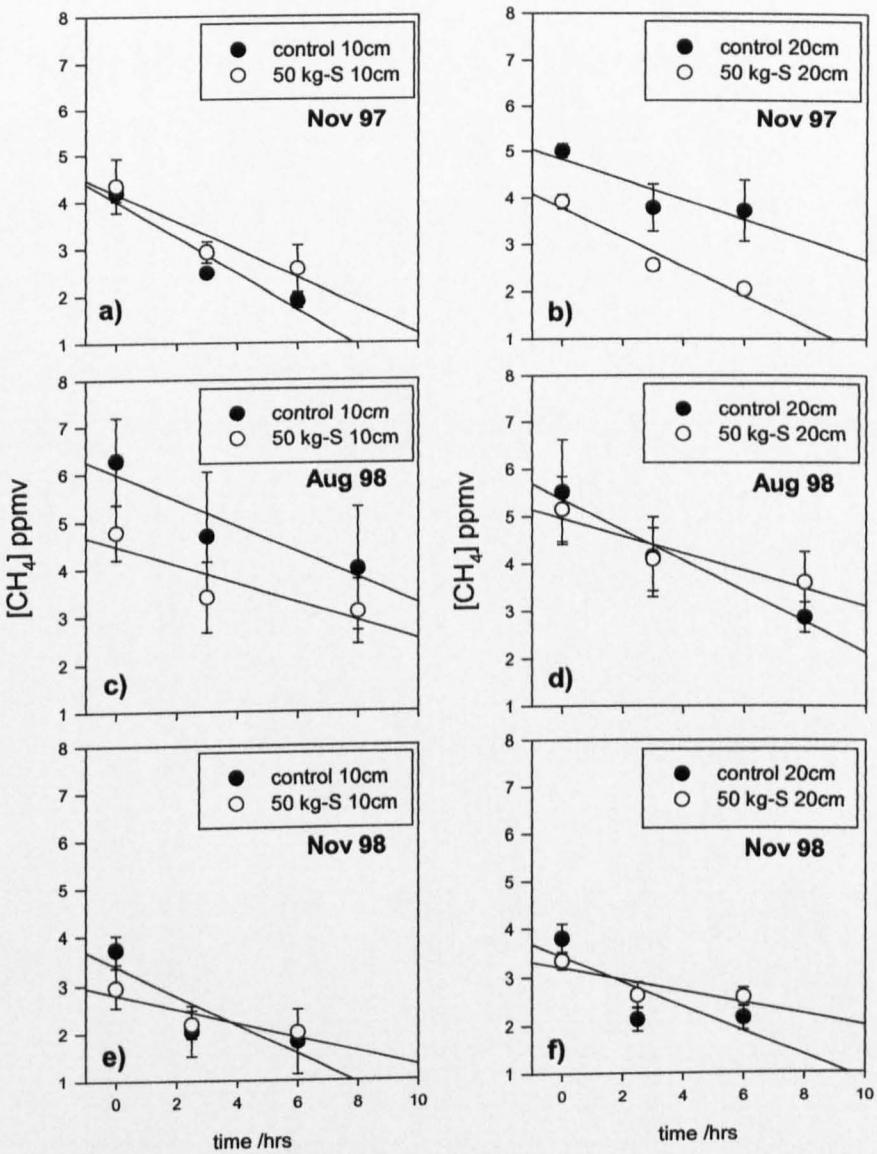

Figure 4.3: Comparison of rates of aerobic CH_4 uptake for 2 depths (10 and 20cm below the peat surface. Error bars indicate ± standard error of the mean (n=3). All regressions are significant ($P<0.05$).

4.4 Conclusions

Results from this experiment show that after 18 months of small regular additions of SO_4^{2-}, at rates equivalent to that experienced in regions affected by acid rain (50 kg-S ha^{-1} yr^{-1}), sulfate reduction potential was significantly larger (up to10 times larger) than in control plots where ambient S deposition rate is one-tenth that of treatment plots. This increase in SO_4^{2-} reduction potential may suggest the presence of an enlarged population of SRB in plots where CH_4 emissions were significantly reduced by 30-40%.

These data suggest that similar studies at higher temporal and spatial resolutions should take place in order to examine more closely the links between SRB population dynamics and CH_4 production in acid rain-impacted wetlands. These synoptic data do, however, present strong evidence that the observed reduction in CH_4 emissions from peatlands experiencing enhanced sulfur deposition loads is due to both a microbial community shift, which favours SRB over methanogens, as well as a functional shift in existing syntrophic SRB.

Chapter Five

Methane emissions and sulfur dynamics in peat affected by simulated 'acid rain' sulfate deposition: Controlled environment experiments

5.1 Introduction

Wetland methane emissions and sulfate deposition through acid rain have only recently been considered as interconnected phenomena (Fowler *et al*, 1995; Dise and Very, 2001; Granberg *et al.,* in press; Chapter 3). As a result there are few studies which have simultaneously investigated both the effect of low but repeated doses of sulfate on CH_4 emissions and the effects of increased S deposition on S fluxes and pools in peatlands. Nor have there been any studies that have investigated the effect of sulfate deposition on the temperature response of CH_4 emission in peatlands although evidence suggests that such an interaction may exist (Bodegom and Stams 1999; Chapter 3). Such studies are of importance as they may provide us with insights on a major controlling variable in peatland carbon biogeochemistry.

Most early work investigating a link between S deposition and suppression of CH_4 emissions involved the application of large single applications of SO_4^{2-} (Fowler *et al* 1995; MacDonald 1998). More recent work has involved the application of numerous small doses of SO_4^{2-} (Dise and Verry, 2001; Granberg *et al.*, in press). It has been

suggested that the intense deposition episodes of SO_4^{2-}, in single dose studies, may be less efficient at lowering CH_4 fluxes than continuous low-level deposition of SO_4^{2-} (Arah and Stephen 1998; Fowler *et al.*, 1995). Through manipulation of S inputs to intact peat monoliths with large individual SO_4^{2-} doses amounting to 40kg SO_4^{2-} S ha^{-1} yr^{-1}, Fowler *et al* (1995) found that emissions decreased over time in treated plots reaching a maximum 40% suppression relative to controls after around three weeks. This was followed by a period of recovery, where treated monoliths reach pre-treatment levels after about 4 additional weeks. Such large single pulses of SO_4^{2-} may stimulate 'boom - bust' cycles amongst sulfate-reducing bacteria (SRB) populations, prompting rapid recovery of methane fluxes several weeks after the treatment application as the labile SO_4^{2-} pool decreases (Arah and Stephen 1998). SO_4^{2-} may be lost either through gaseous emission from the peat as volatile reduced sulfur compounds or through conversion to biologically recalcitrant forms. Through the application of regular small pulses of SO_4^{2-} it is likely that the continuous supply of low levels of SO_4^{2-} may maintain SRB at elevated, competitive levels (Dise and Verry accepted). The two modes of SO_4^{2-} deposition have not, however, prior to this study been directly compared.

5.2 Methods

5.2.1 CH_4 fluxes from peat monoliths

A detailed description of the methods used in to collect peat monoliths, the experimental treatments applied and the methods used to measure CH_4 fluxes is given in Chapter 2 section 2.6.

5.2.2 Gaseous S fluxes

The flux of sulfur containing trace gases emitted from the peat monoliths was examined during sampling for CH4, Tedlar bags were filled with air drawn from the headspace using a 1L gas syringe during the incubation period at on Julian days 89, 99, 106 and, prior to the end of the experiment, day 141. The air contained within each bag was analysed using flame photometry in a Meloy SA285 FPD analyser within 15 minutes of being collected.

5.2.3 Temperature Response

At the end of the manipulation experiment a temperature response experiment was carried out on all 24 monoliths to investigate in closer detail the observed interaction between temperature and SO_4^{2-} treatment effect on CH_4 fluxes (*Chapter 3*). Flux measurements continued as described above, but with temperature altered by successively increasing CONVIRON temperatures from 5 to 10, 15 and finally 20 °C for 24 hours at each temperature. Previous experiments using the same experimental materials and procedures showed that entire monoliths equilibrate to a change in

temperature with 15 hours (*Macdonald et al., 1998*). This was verified by using a thermocouple probe at various depths prior to measurement of CH_4 fluxes.

5.2.4 Chemical analysis of sulfur forms in peat and vegetation

On completion of the temperature response experiment the monoliths were moved back to the glasshouse where water table and SO_4^{2-} treatments were maintained for a further 3 months. The control monoliths and monoliths treated with the small doses of SO_4^{2-} amounting to a rate of 50 Kg-S $ha^{-1}yr^{-1}$ were sampled for analysis of reduced S species in the peat and for total S and dissolved SO_4^{2-} in the above surface vegetation. A square sectioned corer (5cm x 5cm) was used to remove a peat core to a depth of 20 cm from each of the 8 monoliths (4 replicates). A 2.5g sample of peat for analysis of reduced S species was removed from each core at a depth of 10cm below the peat surface/water

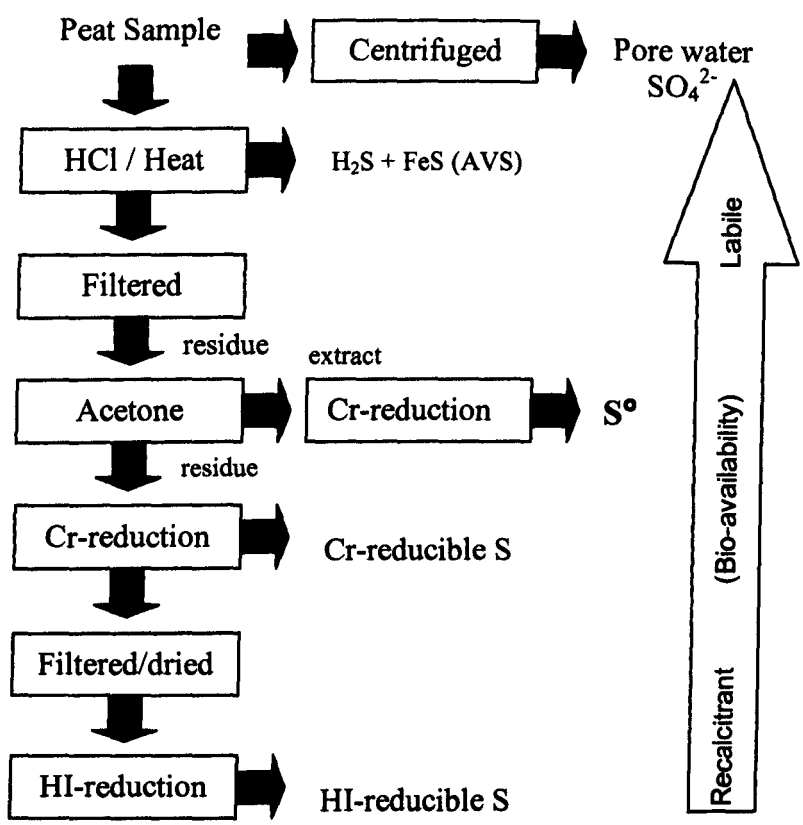

Figure 5.1. Schematic of sequential analysis of S forms in peat and the relative bioavailability of the fractions (after Chapman in press).

table. The remaining core was subdivided into 4 x 5 cm sections for analysis of pore water SO_4^{2-} which was determined by centrifuging peat from each section (3600rpm for 30 min.), filtering the centrifugate (Whatman No. 37) and analysis by ion chromatography (Dionex). The 2.5g peat samples were analysed for reduced S forms by sequentially reducing peat samples using a standard Johnson and Nishita (1952) distillation apparatus using methods reported in Wieder *et al* (1985) and Chapman (in press) (fig *5.1*). To measure Acid-volatile S (AVS) 10ml of 6M HCl was first added to each peat sample, and the mixture gently distilled for 40 minutes under N_2. Volatilized H_2S from the distillation was trapped in a zinc acetate-sodium acetate solution and determined colorimetricaly using the methelyne blue reaction (Johnson and Nishita, 1952) against the product of similarly digested (HI reduction) Na_2SO_4 standards. Residues from the AVS reductions were washed (with deionised water) and filtered to remove sulfate on a Whatman GF/F filter while under suction, after which residues were extracted with 20ml acetone in conical flasks overnight (rotary shaker at 20°C). This mixture was then filtered to separate the extract-laden acetone from the residue (Whatman No. 1) and the acetone allowed to evaporate from both filtrate and residue in preparation for the next reduction step. Elemental S was measured by reducing the dried extract with 8ml of freshly reduced $CrCl_3$ solution ($\rightarrow$Cr(II)) (Zhabina and Volkov, 1978) and 4ml of concentrated HCl, again over gentle heat for 40 min. Trapped sulfides were determined as for AVS determination. Cr-reducible S, of which a large fraction is S in the pyrite form, was measured by distilling the dried residue as for elemental-S. Following this reduction the residue was filtered under suction (Whatman GF/F) and washed with deionised water, oven dried for 12 hours (105°C) and finely ground. 250mg of this finely divided residue was then subjected to the final HI reducing step (Johnson and Nishita, 1952) in order to measure ester SO_4, or "HI reducible S". Total S at 10cm depth was determined using X-ray fluorescence

spectroscopy and C-bonded S was determined as the difference between total S and the sum of the measured fractions of S (Chapman 2001).

The amount of S in the monolith vegetation was also quantified. Around 30g of above surface, non-*Sphagnum sp*, vegetation was removed from each of the analysed monoliths, which mainly consisted of sedges (*Trichophorum cespitosum and Eriophorum sp.*). Total S was measured as for peat and dissolved intracellular SO_4^{2-} was assessed by firstly drying the plant matter at 80°C for 12hrs. The material was then finely milled and 250mg from each monolith sample was placed into a conical flask with 25ml of distilled water. The flasks were then shaken on a rotary shaker for 2 hours and then filtered (Donald 1994). The filtrate was then analysed for sulfate by ion chromatography (Dionex).

5.2.5 Statistical analysis

Methane fluxes are expressed as in ng m^{-2} s^{-1}. The total mass of methane emitted during the experiment was calculated by integrating flux measurements over time. Post treatment CH_4 fluxes were adjusted by the pre-treatment to designated treatment mean flux ratio. Treating the post-treatment data in this manner allowed between treatment variability to be taken into account prior to statistical analysis. Treatment effects on CH_4 fluxes were determined by repeated measures multivariate analysis of variance (MANOVA) (MINITAB, release 11, Minitab inc. USA) with sampling time points included as a variable. This method permitted the evaluation of within-treatment variability (time effect and interactions between time and treatment (time x treatment)). Temperature response Q_{10} values were calculated using equation 5.1, where t_1 is the

upper limit of the temperature range (°C), t_2 is the lower limit and y_1 and y_2 are the CH_4 fluxes at t_1 and t_2 respectively.

$$Q_{10} = \left(y1/y2\right)^{10/(t1-t2)} \tag{5.1}$$

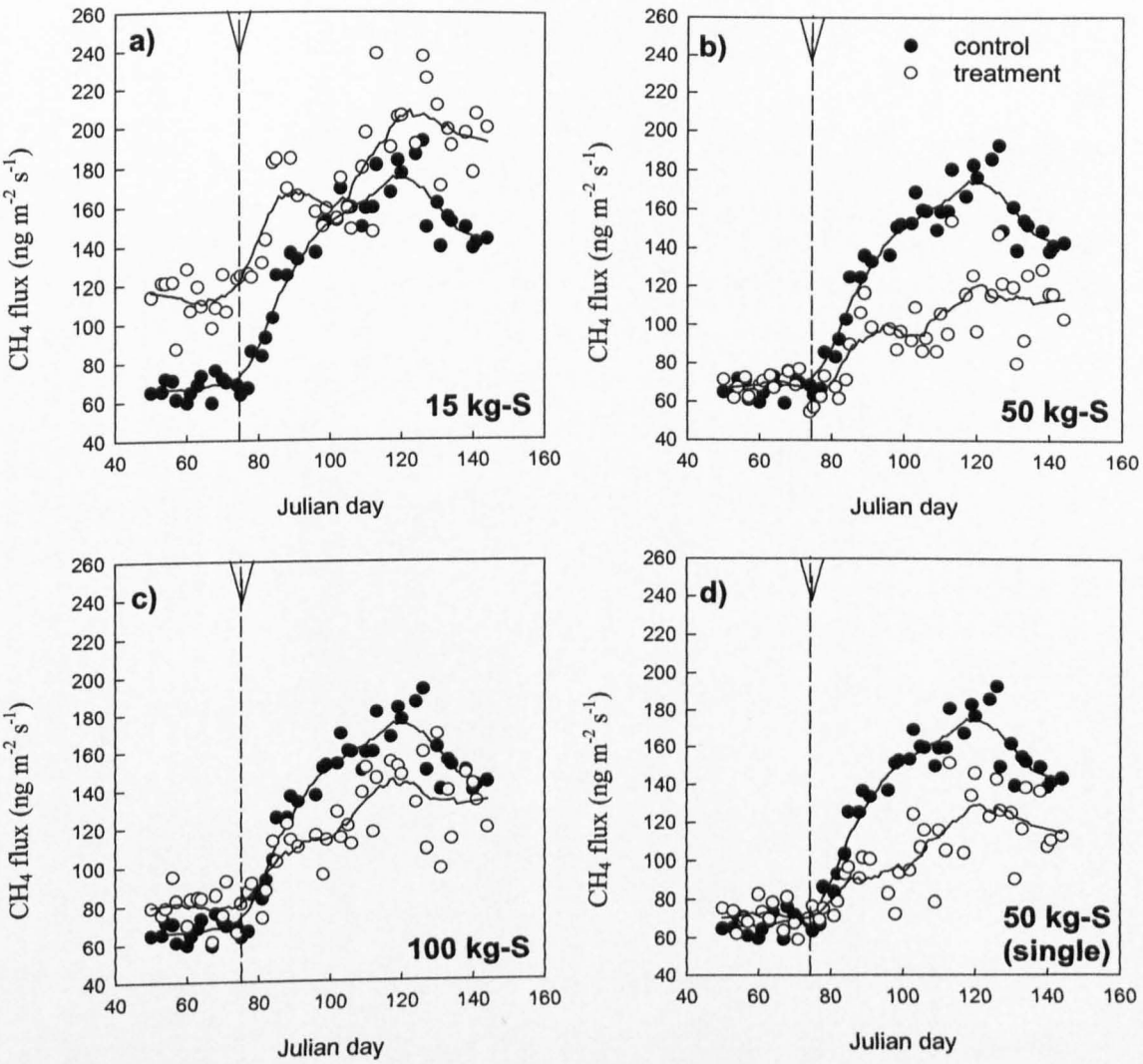

Figure 5.2: Measured methane emissions from CONVIRON experiment. Each point denotes the mean CH_4 flux from each treatment and control (n=4). Dashed line and arrow indicates the start of treatment applications. In the case of the 50 kg-S (single) treatment, this indicates the date of the single large dose application. Trend lines are moving data averages.

5.3 Results

5.3.1 CH_4 fluxes

Methane fluxes from the experiment were stable prior to the start of the treatment additions at around, 110 ng m^{-2} s^{-1} in the monoliths designated for the 15 kg-S treatment and around 70-80 ng m^{-2} s^{-1} in all other monoliths. This difference in the 15 kg-S treatment is possibly due to one or more relatively high emitting monoliths having been assigned, by chance to this treatment. Soon after the onset of the treatment

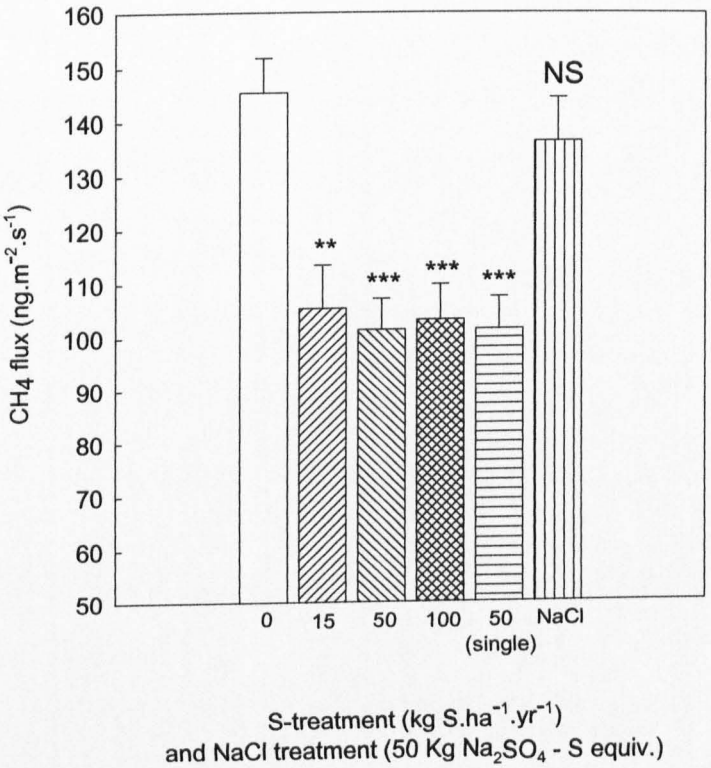

Figure 5.3: Average post-treatment CH_4 flux from treatments and control. Fluxes were corrected for pre-treatment differences as detailed in section 5.25. Error bars indicate standard error.
** = P= 0.001, ***= P<0.001,
NS = P>0.05

applications, emissions increased until reaching a maximum flux (of around 190 ng m^{-2} s^{-1} for the controls) by day 125, around 50 days later (fig. 5.2). Treated monoliths showed a similar CH_4 flux pattern although the rate at which fluxes increase were markedly reduced relative to control monolith fluxes soon after the start of treatment applications (fig. 5.2). Although no detailed measurements of vascular plant biomass were made during the experiment, new vascular plant shoots within the monoliths were observed to have grown considerably, and reached seeding stage (*Eriophorum* spp.) during this period of CH_4 flux growth. After day 125 all treatments and control exhibited a marked decrease in emission rates although this was more pronounced in the controls than in the treatments (fig. 5.2 a-d).

5.3.2 Treatment effects on CH_4 emissions.

Results from the different treatments are summarised in figure 5.3 and table 5.1. After correcting for pre-treatment, between treatment differences (section 5.2.5), CH_4 emissions from all three continuous low dose SO_4^{2-} treatments and the single large 50kg SO_4^{2-} treatment averaged around 100ng m^{-2} s^{-1} (fig 5.3). Mean fluxes were around 30% lower than controls, which averaged 145 ng m^{-2} s^{-1} (n= 136 RM-MANOVA, $P < 0.01$ in all cases; fig. 5.3; table 5.1). There were no observed significant differences in CH_4 fluxes between the different SO_4^{2-} treatments ($P>0.05$).

CH_4 fluxes from the NaCl treatment (50kg $SO_4^{2-}ha^{-1}yr^{-1}$ equivalent) averaged 6% less than control fluxes during the post treatment period although this difference was not significant (n=136 RM-MANOVA, P=0.55). The 50kg $SO_4^{2-}ha^{-1}yr^{-1}$ continuous, treatment also gave significantly smaller mean fluxes than the equivalent ionic strength treatment of NaCl ($P = 0.014$) although there was no statistical difference in the mean flux between the two modes of SO_4^{2-} deposition (single dose versus continuous small weekly pulses, $P = 0.979$.).

TREATMENT	Mean CH_4 Flux (±s.e.) (ng CH_4 .m^{-2}.s^{-1})			P-value *(Control vs. Treatment)*
Continuous-treatment (day 77 – day 144)	n = 136	difference from control %	Total flux (mg/m^2)	
Control	**145 (6.3)**		**12.6**	
15 kg SO_4^{2-}S. ha^{-1}.yr^{-1}	105 (8.0)	-28	9.1	0.001
50 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	101 (5.8)	-31	8.8	< 0.001
100 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	103 (6.6)	-29	8.9	< 0.001
50 kg SO_4^{2-}-S. ha^{-1} (single)	102 (6.0)	-31	8.8	< 0.001
NaCl (50 kg SO_4^{2-}-S. ha^{-1}.yr^{1} equivalent.)	136 (8.1)	-6	11.8	0.55
NaCl and single dose-treatment against continuous 50kg-S (day 77 – day 144)		difference from 50 kg cont. %	Total flux (mg/m^2)	P-value (50 kg S (cont.) vs. Treat)
50 kg SO_4^{2-}-S. ha^{-1}.yr^{-1}	**101 (5.8)**		**8.8**	
50 kg SO_4^{2-}-S. ha^{-1} (single)	102 (6.0)	0	8.8	0.979
NaCl (50 kg SO_4^{2-}-S. ha^{-1}.yr^{1} equivalent.)	136 (8.1)	+26	11.8	0.014

Table 5.1: Summary table of average methane emissions during the post-treatment period. Fluxes were corrected for pre-treatment differences as detailed in section 5.25. P-values indicate significance of treatment (treatment vs. control) effect on mean daily flux as evaluated from repeated measures MANOVA (Wilk's Lambda test). No significant 'time x treatment' interactions were observed. The total post treatment flux of CH_4 was calculated by integrating the mean daily flux over time.

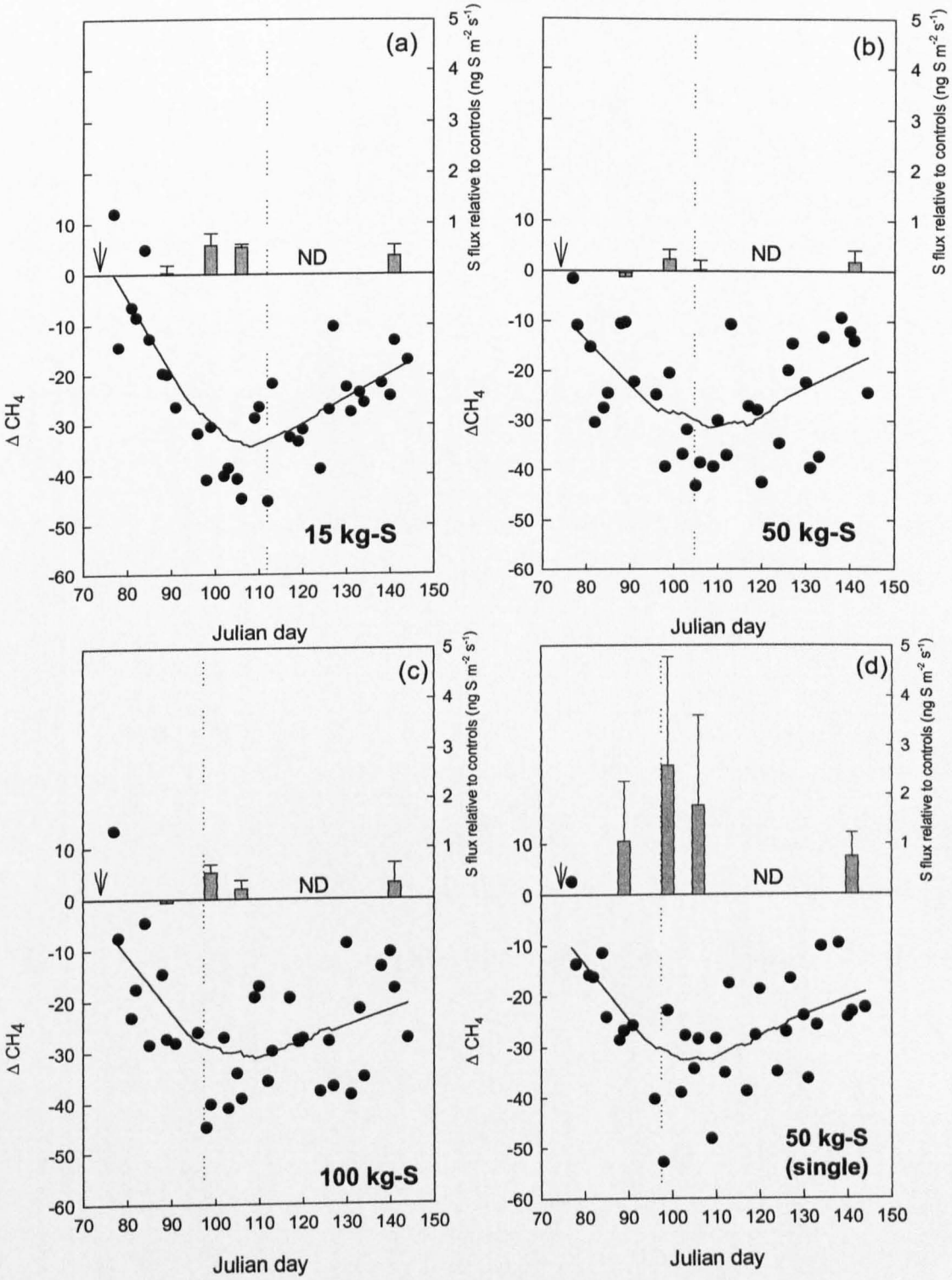

Figure 5.4: Relative change in CH_4 and volatile sulfur compound (VSC) fluxes. Percentage change in CH_4 flux relative to CH_4 flux from controls (ΔCH_4 calculated from equation 5.1) i.e. negative values indicate a relative suppressive treatment effect. Trend lines indicate moving average of 3 time points. Bars represent relative difference in VSC flux between treatment and control on 4 sampling days. Error bars represent ± 1 standard error. The vertical dotted line identifies the time at which the strongest suppression was measured.

The extent and variability in the SO_4^{2-} treatment effect over time was estimated by taking the proportional control to treatment pre-treatment difference as the best estimate of background variability and then estimating the relative (percentage - ΔCH_4) extent to which the treatment flux was lowered over the course of the experiment (chapter 3 section 3.4.3).

In all SO_4^{2-} treatments (continuous and single application experiments), a progressive increase in the suppressive effect of SO_4^{2-} on treatment CH_4 fluxes relative to control flux was observed. Maximum negative ΔCH_4 for each treatment was reached earliest in both the 100 kg-S and the 50 kg-S (single) treatments (day 98, fig. 5.4). The continuous 15 and 50kg-S treatment reached their lowest ΔCH_4 on days 112 and 105 respectively, demonstrating a dose response in the rate at which the maximum SO_4^{2-} effect is reached. Maximum percentage suppressions in each treatment were for the 15, 50, 100 and 50 single kg-S $ha^{-1}yr^{-1}$ treatments, 46%, 46%, 47% and 55% respectively. After reaching the maximum level of suppression differences between control and treatment fluxes decreased from all SO_4^{2-} treatments monoliths until the suppression reached around 20% for all experimental monoliths at the end of the experiment (day 144, fig 5.4)

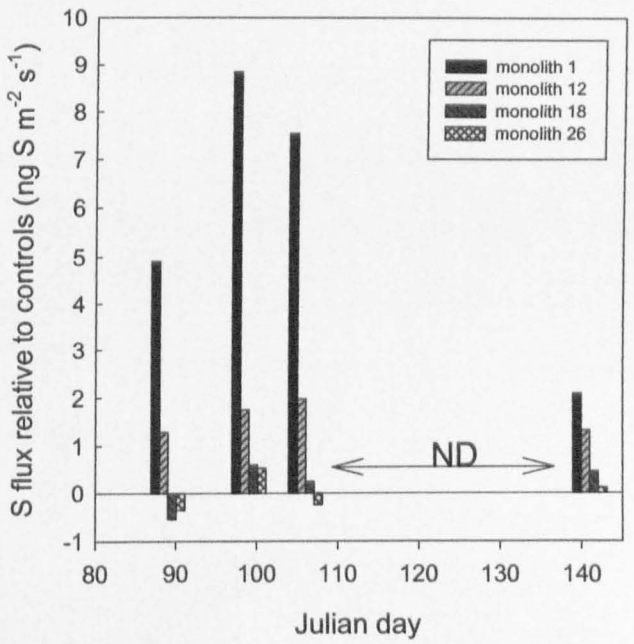

Figure 5.5: Volatile sulfur compound flux from 50kg-S (single) treatment monoliths. Columns represent flux from individual monoliths. All four monoliths were sampled on the same day.

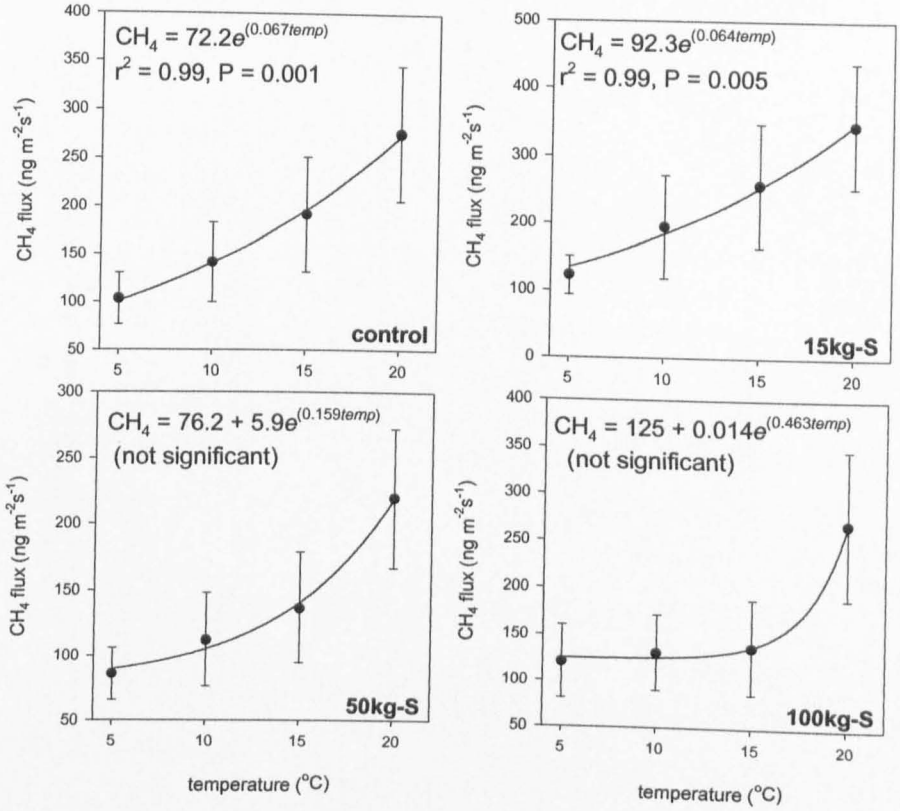

Figure 5.6: CH_4 flux response to changes in temperature. Each point represents the mean of measurements from 4 monoliths in each treatment. Error bars indicate ± 1 standard error. Trend lines represent a 'best fit' of means (with regression equations).

5.3.3 Gaseous S fluxes

Analogous to the differences calculated in CH_4 fluxes, fluxes of volatile sulfur containing compounds (VSC) from treatments relative to controls were assessed by taking the difference between mean control and treatment fluxes. VSC flux differences were generally largest on day 99 (the second VSC sampling day) although mean flux differences amounted to less than 1 ng m^{-2} s^{-1} in all continuous SO_4^{2-} treatments. In the single 50 kg-S $ha^{-1}yr^{-1}$ addition of SO_4^{2-} treatment, two monoliths exceeded 1 ng m^{-2} s^{-1} on all four sampling days (monoliths 1 and 12) with monolith 1 giving the highest fluxes of S in the experiment (up to 9 ng m^{-2} s^{-1} on day 99) (fig. 5.4). Estimated total, time-integrated VSC fluxes (relative to controls) from the three continuous treatments were, in order of increasing dose strength, 2.0, 0.4 and 1.1 mg S m^{-2}, and 9.2 mg S m^{-2} in the single application treatment.

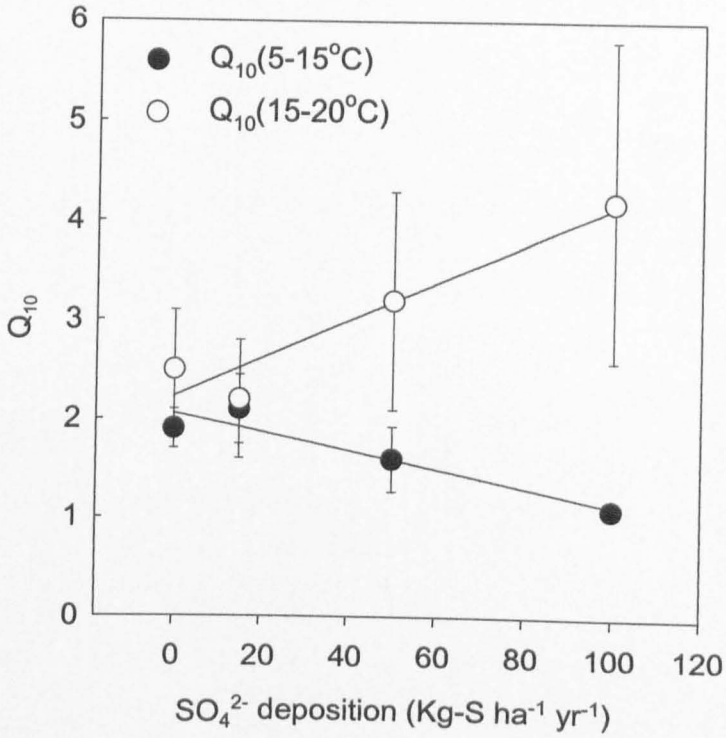

Figure 5.7: Effect of increasing rates of sulfate deposition on the response of CH_4 fluxes to changes in temperature (Q_{10}) over two temperature ranges (5-15°C and 15-20°C). Error bars are ± 1 standard error of the mean. Regression lines were calculated from the 4 mean values for each temperature range only.
Q_{10}(5-15°C) = 2.1 – 0.0093(S-dep), r^2 = 0.89, P = 0.05
Q_{10}(15-20°C) = 2.2 + 0.0039(S-dep), r^2 = 0.92, P = 0.04

5.34 CH_4 temperature response

CH_4 emissions increased exponentially with successive increases in temperature between 5 and 20°C. This relationship was highly significant (P<0.01) in both control and 15 kg-S treatments however an additional term was required to fit the relationship in both the 50 and 100kg-S treatments (P>0.05)(fig. 5.6). The two higher dose rate treatments exhibited a smaller temperature response at lower temperatures than do the controls and 15 kg-S treatments. Analysis of Q_{10} values from the different SO_4^{2-} treatments for two different temperature ranges (5-15 and 15-20°C) illustrates the differences in temperature response, which are related to SO_4^{2-} deposition (fig. 5.7). The two temperature ranges were chosen as CH_4 emissions from monoliths receiving large S treatments responded very differently at temperatures higher than 15°C than they did at temperatures below 15°C (fig 5.6). In the 100 kg-S treatments, Q_{10} values were significantly lower in the 5-15°C range than in the 15-20°C range (One-way ANOVA,

P=0.013, n=4). Linear regression lines fitted to the Q_{10} values for each temperature range show that temperature response is negatively correlated with an increase in SO_4^{2-} deposition in the cooler temperature range but changes to a positive correlation at temperatures in excess of 15°C. These regression equations of mean Q_{10} values were statistically significant (fig. 5.7).

5.3.5 Sulfur forms in peat and vegetation.

Sulfur form	Control		50 kg-S		P-value (of mass comparison)
	μgSg^{-1} dry wt (± SE)	(%)	μgSg^{-1} dry wt (± SE)	(%)	
Sulfate	24 (2)	(1.0)	12 (2)	(0.6)	0.009**
Acid Volatile S	16 (7)	(0.7)	8 (3)	(0.4)	0.236
Elemental S	19 (3)	(0.8)	8 (2)	(0.4)	0.020*
Cr-reducible S	105 (29)	(4.5)	123 (41)	(5.8)	0.730
HI-reducible S (organic)	575 (149)	(24.4)	647 (179)	(30.6)	0.763
C-bonded S (organic)	1616 (207)	(68.6)	1318 (177)	(62.3)	0.303
Total inorganic S	165 (34)	(7.0)	151 (44)	(7.2)	0.811
Total organic S	2190 (90)	(93.0)	1966 (60)	(92.8)	0.029*
Total Sulfur	2355 (75)	(100)	2118 (81)	(100)	0.076

Table 5.2: Sulfur forms at 10cm below the peat surface in mass per g of dry peat (± 1 standard error, n=4) and as a percentage of the total S contained within the peat. Between treatment comparisons were made using analysis of variance.* P<0.05, **P<0.01

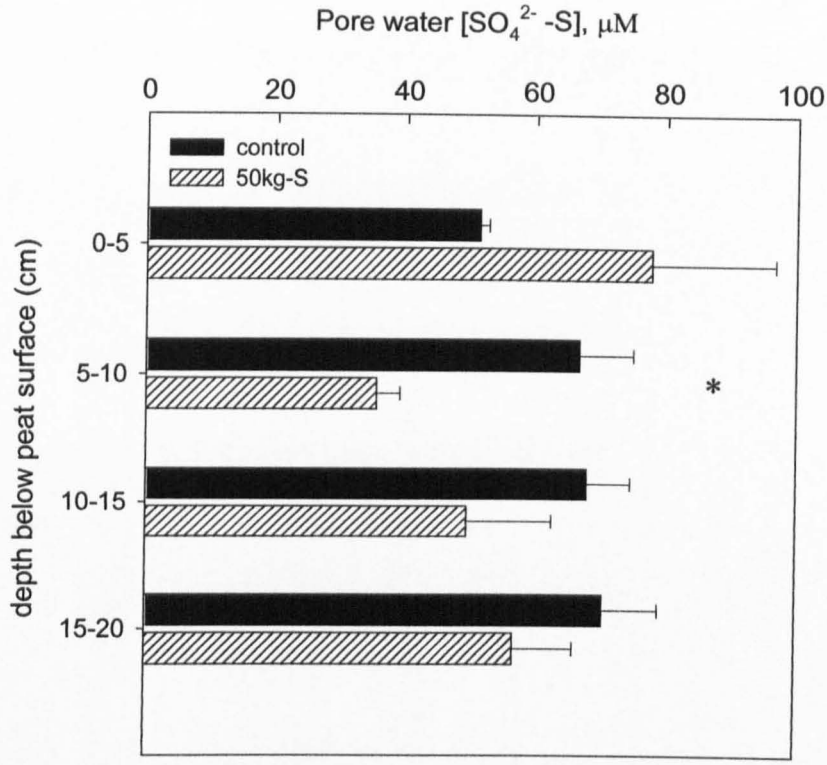

Figure 5.8: Pore-water sulfate concentrations at 4 depth ranges in control and treated monoliths. Error bars indicate ±standard error. *P<0.05 ANOVA

The fractional composition of sulfur forms in peat from both the control monoliths and monoliths treated with a Na_2SO_4 treatment of 50kg -S $ha^{-1}yr^{-1}$ (2.5g S m^{-2} over the 6 month treatment period) are presented in table 5.2. The pools of total organic S(TOS) (C-bonded S + HI reducible S (ester sulfate)) in the two treatments were statistically different (P<0.05) amounting to a 10% smaller pool in the SO_4^{2-} treatment. There was, however, no difference in terms of the overall percentage of the TOS fraction within samples from each of the two treatments (93%). The largest fractions in peat from both treatments was C-bonded S followed by ester-SO_4^{2-}(HI-reducible S) and then Cr-reducible S which formed the largest of the inorganic S fractions. Total sulfur was 10% smaller in the SO_4^{2-} treated monoliths, although this difference was not significant (P=0.076).

Analysis of inorganic fractions, by analysis of variance, yielded significant differences, between control and treated monoliths, for both the SO_4^{2-} (P<0.01), elemental S (S^o)

(P<0.05) pools. In both these cases the SO_4^{2-} and elemental S pools were around 50% smaller in the monoliths treated with SO_4^{2-} than in the controls. The mean AVS fraction was smaller by the same percentage in the treated monoliths than in the controls but this difference was not statistically significant (P>0.05).

Correlation analysis (Pearson, n=4) between different S fractions in the treated monoliths showed negative correlations between Cr-reducible S and pore water sulfate concentrations in the surface peat layer (0-5cm below the peat surface)(R = -0.969, P<0.05). Ester SO_4^{2-} and Cr-reducible S in the treatment monoliths were positively correlated (R = 0.989, P<0.05), as were total inorganic sulfur (TIS) and ester SO_4^{2-} (R = 0.998, P<0.01). In the controls only pore water SO_4^{2-} (at 10cm below peat surface) and Cr-reducible S were significantly correlated (R = -0.952, P<0.05).

Herbage S form	Control μgSg^{-1} dry wt (± SE)	50 kg-S μgSg^{-1} dry wt (± SE)	P value (ANOVA)
Total S	1815 (452)	3408 (606)	0.05
SO_4^{2-} -S extract (cold water)	289 (102)	1528 (341)	0.01
Organic S	1526 (965)	1879 (645)	n.s.

Table 5.3: Cold water extracted SO_4^{2-} and organic S in herbage from control and SO_4^{2-} treated peat monoliths.

Concentrations of dissolved SO_4^{2-} in plants from the treated monoliths were five times larger than in control monoliths (ANOVA P=0.01, n=4) accounting for the large difference in total S between the two treatments (P=0.05)(table 5.3). There were no significant differences between the organic S fractions in plants from the two treatments.

5.4 Discussion

5.4.1 CH_4 fluxes

Although environmental conditions, including temperature, water table position, day length, light intensity and relative humidity, were maintained at constant levels throughout the 3-month experimental period, CH_4 emissions varied substantially over time (fig. 5.2). In the period following the start of the treatment applications, during a period of observed vascular plant growth, methane fluxes increased with time, e.g. for the controls, from a baseline of 60-70 ng m^{-2} s^{-1} to 180-190 ng m^{-2} s^{-1} at around day 120.

The maintenance of constant environmental conditions excludes temperature change or water table fluctuation as driving variables of CH_4 flux change. As plants were observed to be growing during this period, reaching their peak growth stage, it is likely that this changing variable will have affected emissions as has been found in other natural wetland systems (Kim *et al.*, 1999). Two possible mechanisms may explain this observation: (1) elongation of plant shoots provided a greater leaf area, increasing rates of photosynthesis and, in doing so, stimulating the production of root exudates which will have boosted CH_4 production, and (2), increased root surface area associated with plant growth promoted the exchange of dissolved CH_4 from interstitial waters into plant aerenchymal tissue, thereby promoting CH_4 emission (King *et al.*, 1998). There is also evidence that the root porosity of wetland plant roots may increase as new roots grow, thereby further facilitating root/pore water gaseous exchange and aerenchymal CH_4 transport, (Singh *et al.*, 1998). In addition, the number of shoots may have increased during this growth period, which, in addition to the already stated mechanisms, will have increased the relative contribution of aerenchymal transport to CH_4 efflux (Schimel 1995). Reduction in emissions after the peak emission period (day 125) is again likely

to be associated to physiological changes in the sedges with the onset of senescence. Browning of sedge shoot tips was observed during this period, but the extent of senescence in vascular plants within the experimental monoliths was not quantified.

5.4.2 Treatment effects on CH_4 emissions

In all sulfate treatments, CH_4 fluxes were significantly smaller than in the control monoliths, having been reduced by an average value of around 30%, as were total emissions of CH_4 over the course of the experimental manipulation (fig. 5.3; table 5.1). The annual deposition rate in the smallest SO_4^{2-} treatment was the equivalent of 15 kg ha^{-1} yr^{-1}, or a weekly application rate of 0.29 kg ha^{-1} (or 29 mg m^{-2} $week^{-1}$) a far smaller application rate than in comparable SO_4^{2-} amendment experiments (Dise and Verry accepted, Chapter 3) although a rate of deposition similar to that experienced in many areas of Europe, North America and Asia.

The lack of any significant differences between CH_4 fluxes from the different rates of SO_4^{2-} deposition indicates that the suppressive effect of SO_4^{2-} deposition is not limited by sulfate availability within this deposition range (15-100 kg SO_4^{2-} -S ha^{-1} yr^{-1}) and that the maximum suppressive effect of SO_4^{2-} on CH_4 production is reached at, or below the lower end of the range of SO_4^{2-} deposition rates applied in this experiment. Indeed, the addition of 50kg SO_4^{2-} -S ha^{-1} (5g m^{-2}) in a single application had no additional suppressive effect on average CH_4 fluxes over the smaller application rates.

While a small reduction in average CH_4 emissions was observed in the NaCl treated monoliths over the course of the experiment (mean of 6%), this reduction was not statistically significant (P=0.55). Therefore, by maintaining a continuous addition of NaCl (weekly small pulses of an equivalent ionic concentration to the continuous 50 kg SO_4^{2-} -S applications), the possibilities that a) the suppressive effect of Na_2SO_4 salts on

CH_4 fluxes was due to the increase of the ionic concentration of peat pore water (Nesbit and Breitenbeck 1992), whereby methanogens would became adversely affected by elevated osmotic potential, or b) that the accompanying cation (Na^+) may have affected CH_4 emissions, could both be eliminated.

Analysis of the temporal variability in CH_4 emissions from SO_4^{2-} treatments relative to control fluxes (ΔCH_4, fig 5.4) shows that there is a linear progressive decrease in fluxes from treatments immediately following the onset of treatment applications. The time taken to reach the maximum level of suppression apparently decreased with an increase in SO_4^{2-} dose size (fig 5.4). It is possible that the rate at which maximum CH_4 flux suppression is attained is governed by the rate at which added SO_4^2, at a concentration sufficient to stimulate existent SRB to sulfate-reduction, reaches the zone/depth beneath the peat surface of maximum SO_4^{2-} reducing potential. The speed with which maximum CH_4 suppression is reached may therefore be affected by the rate of diffusivity of solutes in waterlogged peat soils rather than by a change in SRB population size.

The finding that there is an apparent 'recovery' of CH_4 fluxes in all SO_4^{2-} treatments relative controls after maximum suppression is reached challenges previous hypotheses regarding how the two modes of SO_4^{2-} application may contrast in their potential for CH_4 suppression over the long-term (Arah and Stephen 1998; Dise and Verry 2001). In a previous field manipulation, it was suggested that while large additions of SO_4^{2-} as an individual pulse may stimulate a boom of SRB populations followed by a crash as SO_4^{2-} is consumed, small pulses of SO_4^{2-} may enable the maintenance of an elevated SRB population (Dise and Verry 2001.). In this experiment, monitoring of CH_4 fluxes ceased two months after the start of the experimental manipulations and at this point all treatments had recovered from a mean peak in suppression of around 30% to a level of

suppression of around 20 % (fig. 5.4). The recovery in fluxes occurred soon after the maximum treatment effect was observed at a time when CH_4 fluxes, in general, were at their largest. With these data, it cannot be shown whether CH_4 fluxes in low, multiple dose SO_4^{2-} treatments eventually returned to pre-treatment levels (relative to controls) or whether a steady state of CH_4 fluxes, at a level that is lower relative to control emission, is achieved. It has, however, been observed in field studies that prolonged small pulse additions of SO_4^{2-}, over several years, has a long-term suppressive effect on CH_4 fluxes (Chapter 3, Granberg *et al.*, in press) although there is a pronounced seasonal variability in the suppressive effect of added SO_4^{2-} on CH_4 fluxes (Chapter 3).

A hypothesis which may explain the apparent recovery in SO_4^{2-} treated CH_4 fluxes is that large production in labile organic matter (in an otherwise biologically recalcitrant organic medium) during the later growth stages of wetland plants, through root exudates and root degradation, provides an excess of substrate which is capable of maintaining both methanogen and SRB populations. In other words, substrates cease to be limiting, and competition (over common substrates) between methanogens and SRB is reduced. Root exudates (Lu *et al.*, 1999) and decomposing roots have been identified as the dominant carbon source in methane production during the ripening stage of rice growth (Neue *et al.*, 1996), with production of exudates also having been strongly linked to increases in SO_4^{2-} reduction activity in natural salt marsh systems. Similar interactions may, therefore, also occur in peatlands.

Alternatively, sudden perturbations in the SO_4^{2-} supply may temporarily affect microbial communities before long-term, steady state conditions become established. Raskin *et al.* (1996) found that such a perturbation of enhanced SO_4^{2-} supply initially stimulated the number of SRB in an anaerobic biofilm reactor. SRB numbers then decreased over the first 50 days before again increasing to a stable elevated population

size after 100 days. The initial increase and then decrease in this system occurred over a similar time scale to the changes observed in the monoliths treated with enhanced SO_4^{2-}. It is therefore possible that the changes reported here, and those reported by Fowler *et al.* (1995), may reflect temporary responses to the enhanced SO_4^{2-} perturbation before long term changes in microbial community structure have achieved stability as may have been the case in the Moidach More study (Chapters 3 and 4).

5.4.3 CH_4 temperature response

In manipulating temperature over a short period of time (1 week), the possibility that any changes in the relative difference between CH_4 fluxes from SO_4^{2-} treatments and controls may have been due to changes in substrate availability (as discussed in section 3.42) could be minimised thereby allowing the investigation of how temperature changes may affect the degree to which CH_4 emissions are suppressed in SO_4^{2-} impacted peatland systems.

Results from the experiment indicate a decline in CH_4 flux response to temperature increases, between 5 and 15°C, as the SO_4^{2-} application rate increased (figs. 5.6 and 5.7). Above 15°C the opposite is true, with higher SO_4^{2-} application rate treatments responding far greater to temperature increases, although, rather than being larger emitters of CH_4 than lower dose treatments, this is more a 'readjustment' of fluxes to levels comparable with the other treatments. This finding is consistent with the hypothesis that competition between SRB and methanogens over substrates is affected by temperature (Nedwell and Watson 1995; Bodegom and Stams 1999, Chapter 3), with low temperatures favouring SO_4^{2-} reduction over methanogenesis and high temperatures favouring methanogenesis.

An alternative explanation for the observed temperature effect is that higher temperatures accelerate turnover/consumption of SO_4^{2-}, thereby depleting peat of the SO_4^{2-} necessary for microbial competition to be maintained (Granberg *et al.*, in press). This may explain the difference in temperature response between the different SO_4^{2-} treatments at temperatures below 15°C, since it is likely that the 100 kg SO_4^{2-} would have a larger pool of available SO_4^{2-} than smaller treatments. This pool would take longer to consume at temperatures up to 15°C, and, judging by the rapid increase in fluxes relative to the other treatments at higher temperatures (mean Q_{10} of 4) methanogenesis may increase at the expense of SO_4^{2-} reduction.

The implications of these findings suggest that while increased S-deposition may have a long-term suppressive effect on CH_4 emissions from northern wetlands, the effect may be reduced by increases in temperature accompanying projected climate change. A further implication is that naturally S-impacted wetlands, such as coastal wetlands or those overlying S rich deposits, e.g. Hudson Bay Lowland (Reeve *et al.*, 1996) and areas of Belize (Rejmankova and Post 1996), may respond very differently to temperature changes associated with climate change, in terms of CH_4 emissions than would wetlands receiving low inputs of S.

5.4.4 Sulfur fluxes and forms:

5.4.4. 1, VSC flux

Fluxes of volatile sulfur compounds (VSC) from the continuous SO_4^{2-} treatment never exceeded 1 ng S m^{-2} s^{-1} more than control fluxes and most frequently averaged less than 0.5 ng S m^{-2} s^{-1} more than controls (fig 5.3). Treatment fluxes were not significantly larger than VSC fluxes from controls with total time integrated fluxes during the

experiment amounting to between 0.4 and 2.0 mg S m^{-2}. Total additions of S in the continuous SO_4^{2-} application treatments during the 2 month flux measurement period amounted to 250, 830 and mg 1670mg S (in the 15, 50 and 100 kg-S treatments respectively). Fluxes of VSC therefore amounted to less than 1% of S inputs in all three treatments. It is likely that much of the reduced gaseous sulfur gases produced through dissimilatory reduction was reoxidised to oxidised sulfur species (e.g. SO_4^{2-}).

It has been proposed that reduced gaseous S products (e.g. H_2S) may be reoxidised near the peat surface (where O_2 concentrations increase) to SO_4^{2-}, particularly during periods of low water table (Freeman *et al.*, 1994). The release of VSCs from the single application treatment monoliths may indicate that O_2 in the surface peat was consumed during reoxidation of reduced S species, thereby allowing reduced S compounds to diffuse from the system. This mechanism should therefore ensure availability of a SO_4^{2-} pool, which would facilitate SO_4^{2-} reduction long after SO_4^{2-} input was made. The single large S application treatment shows a peak in VSC emission on day 98, which corresponds with the period of maximum CH_4 flux suppression and can therefore be interpreted as period of maximum SO_4^{2-} reduction (figs 5.4 and 5.5). Thereafter, while VSC emissions decrease, emissions are still higher than in the other continuous SO_4^{2-} treatments, implying that O_2 was consumed through oxidation of reduced S compounds. The resultant SO_4^{2-} then contributed to maintaining sulfate reduction. This finding may therefore cast further doubt on the possibility that 'recovery' of treated methane fluxes was due to depletion of the SO_4^{2-} pool following bacterial reduction of the large SO_4^{2-} pulse.

5.4.4.2, S forms in peat and vegetation

As measurements of the different sulfur pools were only made at one depth below the peat surface (10cm), a full sulfur budget could not be calculated. The results did however, provide insights on the cycling of S in SO_4^{2-} impacted peat systems.

The fraction of sulfur within each pool of both the control and the 50 kg S (small pulses) treatment were similar to those for peat from an area close to where the monoliths were collected (Chapman, 2001)(table 5.2). At 10cm below the surface, significant differences were apparent in the inorganic fractions of the peat although, counter to expectation, the sulfate concentration and elemental S fraction were significantly smaller in those monoliths treated with SO_4^{2-} by around 50%. The AVS pool (H_2S and FeS), was also smaller in the treatment monoliths, although differences were not significant.

While mean SO_4^{2-} concentrations were larger (but not significantly so) in the surface peat layer (fig. 5.8) the trend of a decrease in SO_4^{2-} concentrations in treated monoliths was also apparent at depths below 10 cm, although these differences were also not statistically significant. This replicates findings from the long-term field experiment in Moidach More (Chapter 3), and as discussed in that earlier chapter, may be due to additional SO_4^{2-} stimulating SRB populations (possibly by decoupling of SRB-methanogen syntrophic consortia (Raskin et al., 1996)) so that they are better able to scavenge available SO_4^{2-}, and in doing so, they may be able to reduce SO_4^{2-} concentrations to a level which is lower in treatment monoliths than is found in controls.

Weider and Lang (1988) have demonstrated that low concentrations of SO_4^{2-} may not necessarily indicate smaller rates of sulfate reduction since rapid turnover of SO_4^{2-} and cycling within the inorganic S pools can ensure a continuous supply of SO_4^{2-} for SRB.

The negative correlation between SO_4^{2-} and Cr-reducible S suggests that much of the SO_4^{2-}, in both control and peat monoliths may, in the short term, be converted to Cr-reducible S (pyrite-S). If anything, there is more Cr-reducible S in the treatment than in the control and so it is likely that this pool is less susceptible to cycling to form SO_4^{2-} and so recycling of other inorganic S fractions may dominate in maintaining SO_4^{2-} availability. Positive correlations between Cr-reducible S and ester sulfate, and TIS and ester sulfate may simply reflect the likelihood that as one S pool increases in a peatland system, others are also likely to.

In above-ground vegetation collected from the monoliths, more than 50% of total S was present in dissolved inorganic SO_4^{2-} form (table 5.3). The finding that there was no significant difference in organic-S fractions between the two treatments suggests that levels of nitrogen may have been too low to allow much of this SO_4^{2-} to be assimilated into plant material. Uptake of SO_4^{2-} in plants has been reported for a wide variety different plants types, for example barley (Gede *et al.*, 1992), tropical legumes (Bell *et al.*, 1995) and rice (Freney et al., 1982). A simple estimate of above-ground biomass (by counting and then drying and weighing a sample of plant shoots from the monoliths) of between 50-200g dr wt m^{-2} means that this pool may account for between 2.5 and 10% of the SO_4^{2-} added during the 6 months application period. In estimating that two thirds of plant biomass is contained in roots and assuming equal distribution of sulfate, as much as 30% of applied sulfate may have been assimilated by vascular plants. Furthermore, the *Sphagnum spp.* S pool was not quantified although it likely that this may also have been an assimilatory SO_4^{2-} sink. The presence of significant inorganic SO_4^{2-} pool in vascular plants may have implications for S cycling and methane production as degrading roots may provide an additional SO_4^{2-} source during the non-growing season.

5.5 Summary

CH_4 fluxes from all SO_4^{2-} treatments were significantly smaller (30%) than in controls through the duration of the experimental period and this reduction in emissions was not due to a 'salt effect'. The total suppression of CH_4 emissions was not dependent on SO_4^{2-} dose at SO_4^{2-}-S deposition rates in excess of 15kg ha^{-1} yr^{-1}. Temporal changes in the extent of the suppressive effect of the sulfate treatment, i.e. an increasing treatment effect followed by 'recovery', was evident in both the single large SO_4^{2-} application and the continuously applied SO_4^{2-} treatments. Volatile sulfur fluxes were larger in the large single SO_4^{2-} application than in controls during this period of recovery, indicating that dissimilatory sulfate reduction may still have been occurring at rates in excess of those in controls, therefore casting doubt on the hypothesis that SRB populations boom and crash when presented with a finite SO_4^{2-} pulse.

It is hypothesised that the reduction in the SO_4^{2-} treatment effect on CH_4 over time may be due to changes in substrate availability associated with physiological changes in vascular plants accompanying growth and senescence, and so CH_4 flux recovery in treatments may be a transitory feature. Alternatively, the period of suppression and recovery may be due to microbial communities experiencing a period of readjustment prior to gaining stability (Raskin *et al,* 1996) where CH_4 production is suppressed in the long term as was found in the long term Moidach More experiment (Chapters 3 and 4).

SO_4^{2-} and elemental S^0 pools were depleted in SO_4^{2-} treatment monoliths which may reflect a stimulated SRB community which accelerates S turnover within inorganic sulfur pools and, in doing so, brings instantaneous SO_4^{2-} concentrations down to levels below those of control monoliths. A significant proportion of the applied SO_4^{2-} may be

taken up, and stored as SO_4^{2-} in plants, and so retention and release of SO_4^{2-} by vascular plants may play an important seasonal role in peatland sulfur cycling.

CH_4 emissions from the different treatments responded differently to changes in temperature, with high SO_4^{2-} treatments responding minimally to increases in temperature (in comparison to controls) within a low temperature range (5-15°C) but recovering to comparable rates at temperatures in excess of 15°C.

Chapter Six

The potential impacts of climate change and global changes in SO_4^{2-} deposition on the contribution of wetlands to the atmospheric methane budget.

(Data from this chapter are included in: The potential impact of global changes in sulfur deposition on the contribution of wetlands and rice agriculture to the atmospheric methane budget. With Elaine Matthews, Bernadette Walter and Dorothy Koch of the National Aeronautics and Space Administration Goddard Institute for Space Studies, New York, USA as co-authors - NASA Planetary Biology Program Report)

6.1 Introduction

The studies presented in Chapters 3 and 5 provide strong evidence of the effect of SO_4^{2-} deposition on CH_4 emissions from wetlands as well as providing insights into variables that may affect the interaction (Chapters 3, 4 and 5). The link between anthropogenically-enhanced SO_4^{2-} deposition and the inhibition of methane flux from anaerobic soils deserves close attention at the global scale as it provides a candidate for a mechanism behind the continuing slow-down in the atmospheric methane growth rate (Chapter 1, Dlugokencky *et al.*, 2001). In addition, knowledge of the likely effect of future human activity on the sulfur cycle may allow improved prediction of future trends in the growth rate of this radiatively and chemically important gas.

Historical records show that the global sulfur balance has been strongly perturbed during the course of the 20th century with global anthropogenic emissions of sulfur exceeding natural emissions (oceanic dimethylsulfide (DMS) and volcanoes) by a factor

of 5 (Rodhe, 1999). On a regional scale, different trends of sulfur emissions and subsequent acidifying deposition have been exhibited by Europe, North America and Asia. In developed regions such as Europe and North America sulfur emissions peaked in the 1970's and 80's and are now declining as abatement measures take effect (Stoddard *et al.*, 1999.). In Asia, a region of extensive rice agriculture, a different scenario is unfolding with rapid industrialisation resulting in the increased emission and deposition of acidifying compounds, mainly oxides of sulfur (Rodhe, 1999). This increase in deposition is already affecting vegetation as critical loads in parts of China are exceeded (Streets *et al.*, 1999).

Projections of population growth and increased energy consumption to meet the needs of rapidly industrialising countries such as China, suggest that problem of S pollution will continue to increase in the first half of the next century (Streets *et al* 1999). These scenarios can be derived through a combination of detailed inventories of global and regional anthropogenic emissions, the use of long-range chemical transport models at the regional scale (Arndt and Carmichael 1995; Robertson *et al* 1995; Ichikawa and Fujita 1995), and estimates of population and economic growth.

Interest in the role of anthropogenically derived tropospheric SO_4^{2-} aerosol in radiative cooling has also lead to the development of simulations of the behaviour of tropospheric sulfur in general circulation models. These models allow the transport, atmospheric chemistry and deposition of acidifying sulfur compounds to be examined over the entire planet (Chin *et al* 1996; Koch *et al* 1999; Stevenson *et al.*, *submitted manuscript*). These advances coincide with the development of models of CH_4 emission from wetland soils that have evolved from empirical relationships between emissions and dependent variables, such as water table and temperature, (Dise *et al.*, 1993), to one-dimensional process-based and climate sensitive models validated against observations

from natural wetlands spanning climatically distinct regions (Walter 1998; Walter and Heimann, 2000). This enables the net wetland contribution to the global methane budget to be estimated with sensitivity to past and predicted changes in climate.

An improved estimate of trends in spatial patterns of S deposition and CH_4 emission from wetland soils allows the examination of this biogeochemical interaction at the global scale. For this chapter, an approach to up-scaling these processes is described and applied to assess the effect of spatial and temporal changes in SO_4^{2-} deposition on the largest global source of atmospheric CH_4. In doing so, estimates of the changing importance of atmospheric SO_4^{2-} deposition on CH_4 emission during both the 20th and 21st centuries, are calculated.

6.2 Methods

6.2.1 Methane emissions from wetlands

Global, spatially disaggregated fields of CH_4 emission from natural wetlands were estimated for the years 1960, 1970, 1980, 1990, 2000, 2030 and 2080. An adaptation of a process-based, climate sensitive model of CH_4 emissions was used to construct global emissions fields (Walter 1998; Walter and Heimann, 2000). This one-dimensional model simulates global CH_4 production and CH_4 oxidation in response to changes in temperature and water table as well as the relative contribution of each transport mechanism (i.e. diffusion, ebullition and plant mediated transport) to CH_4 emission from wetlands.

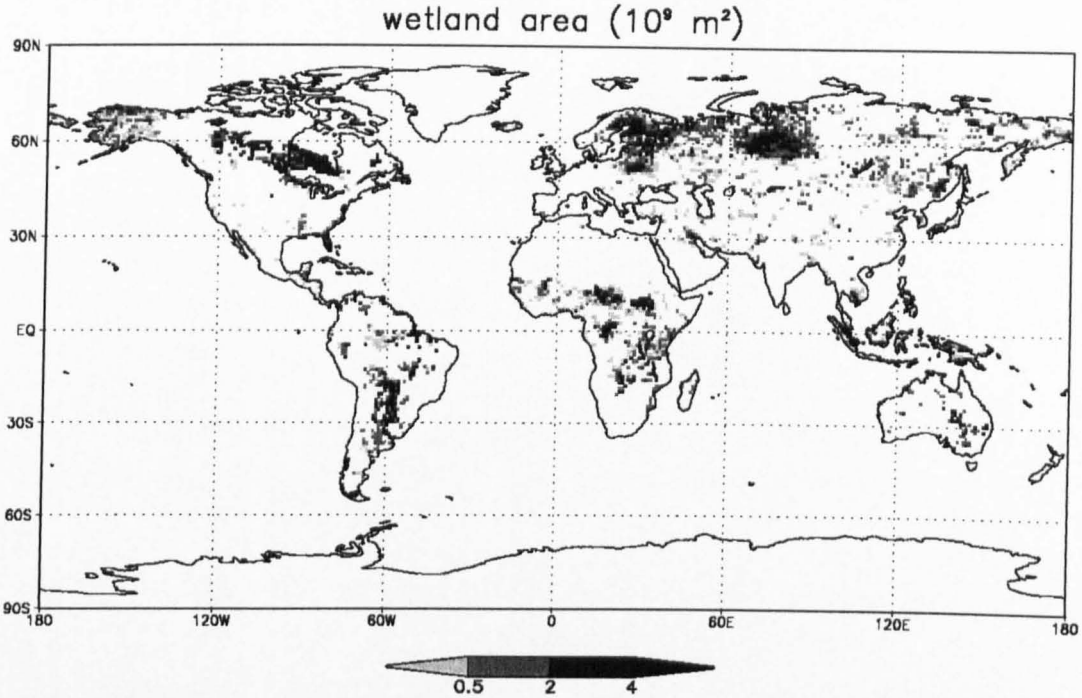

Figure 6.1: Global distribution of natural wetland area (10^9 m^2/ 1°x1° grid cell). (Matthews and Fung, 1987).

The model uses the following input information from the following global data sets: -

- Wetland fractional inundation (Matthews and Fung 1987 fig 6.1).
- Maximum NPP,
- Total annual NPP
- Rooting depth,
- Soil depth,
- Relative soil coarse pore volume,
- Fractional area of unvegetated, bare soil,
- and the ability of plants to conduct gas. (Walter 1998)

Temperature and hydrology were driven by daily climate data (temperature and precipitation) from the European Centre for Medium-range Weather Forecasting (ECMWF reanalysis) in combination with a hydrological model of water movement

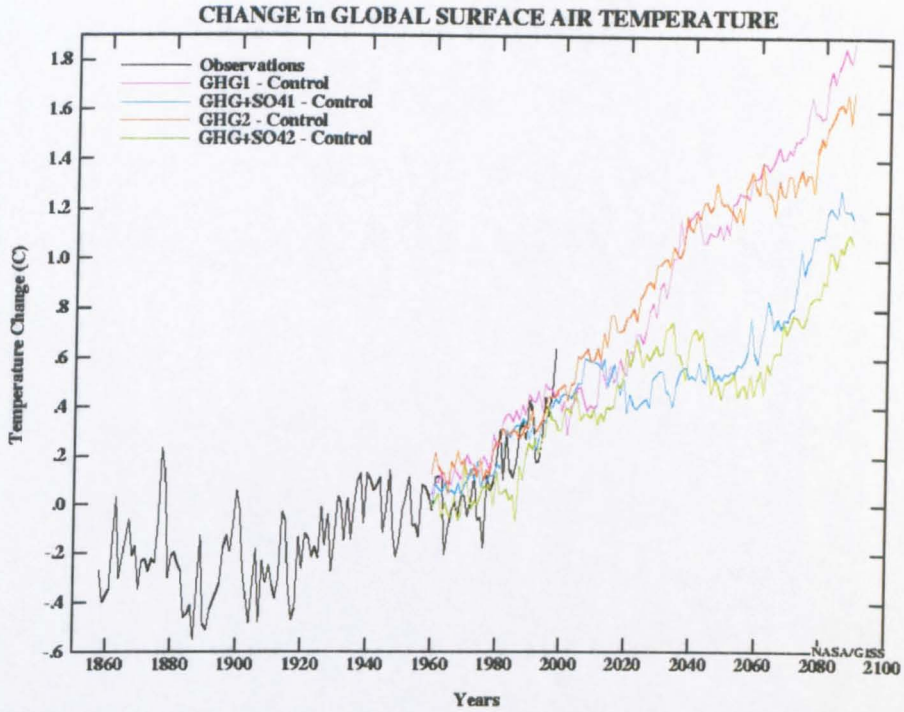

Figure 6.2: Change in global annual surface air temperature (°C) for the observations of Hanson *et al.*, (submitted) and four model experiments (2 scenarios used in this study replicated, i.e. with and without SO_4^{2-} aerosols, minus 21 year averages of control simulations. Anomalies are calculated as the difference from a 30 year (1951-1990) mean (from Russel *et al.*, 2000)

through soil (Walter 1998). The result is a 1°x1° model of daily CH_4 emission from wetlands

The model was validated against data from 5 long-term wetland CH_4 monitoring experiments, located in areas receiving small ambient rates of S deposition (≤5 kg S $ha^{-1}yr^{-1}$), and spanning a range of climate regions from boreal latitudes to the tropics. Results of model runs successfully simulated changes in the global wetland CH_4 source that correspond well with observed changes in atmospheric CH_4 concentrations (Walter 1998; Dlugokenky *et al.*, in press). As detailed by Walter (1998), CH_4 emissions for a 12-year period (1982-1993) were simulated using this model. The adaptation of this model was constructed by first examining the relationship, using multiple linear regression analysis, between modelled monthly CH_4 anomalies and monthly anomalies

of temperature and a two-week lag in precipitation over 12 years for the entire $1^{o}x1^{o}$ gridded data set of natural wetlands as defined by fractional inundation (Matthews and Fung, 1987, fig 6.1). The regression model was found to capture the majority of variability in CH_4 emissions induced through changes in temperature and precipitation ($r^2 = 0.8$ Walter *pers. comm.*).

Using this regression model, new CH_4 anomalies were calculated for the time period being investigated (1960-2080) by using twenty-year running means of two climate scenarios generated by the NASA GISS coupled ocean-atmosphere model as climate input (temperature and precipitation) (Figure 6.2 Russell *et al.*, 2000). One of these scenarios (2 GCM runs of which are illustrated in red and cyan in fig 6.2) estimated the effect of changes in atmospheric greenhouse gas content on climate between 1960 and 2080 (GHG run, fig 6.2) and the second scenario estimated the combined impact of greenhouse gases and the relative cooling effect of atmospheric SO_4^{2-} aerosols on climate (GHG+SO4, blue and green data in fig 6.2; Russel *et al.*, 2000). Spatially disaggregated CH_4 emissions fields were produced at decadal intervals as the mean emission for the year plus and minus 3 years (e.g. average of emissions from 1967-1973 for 1970).

6.2.2 Sulfur deposition model

Global sulfur deposition fields were produced from the tropospheric sulfur simulation in the Goddard Institute for Space Studies General Circulation Model (GISS GCM) (Koch *et al* 1999). In total, seven model runs were used to estimate total sulfur deposition for the years 1960, 1970, 1980, 1990, 2000, 2030 and 2080. The model runs for between 1960 and 1990, were produced from SO_2 emissions data compiled by Lefohn *et al* (1999) and the remaining runs for the years 2000, 2030 and 2080 were produced from

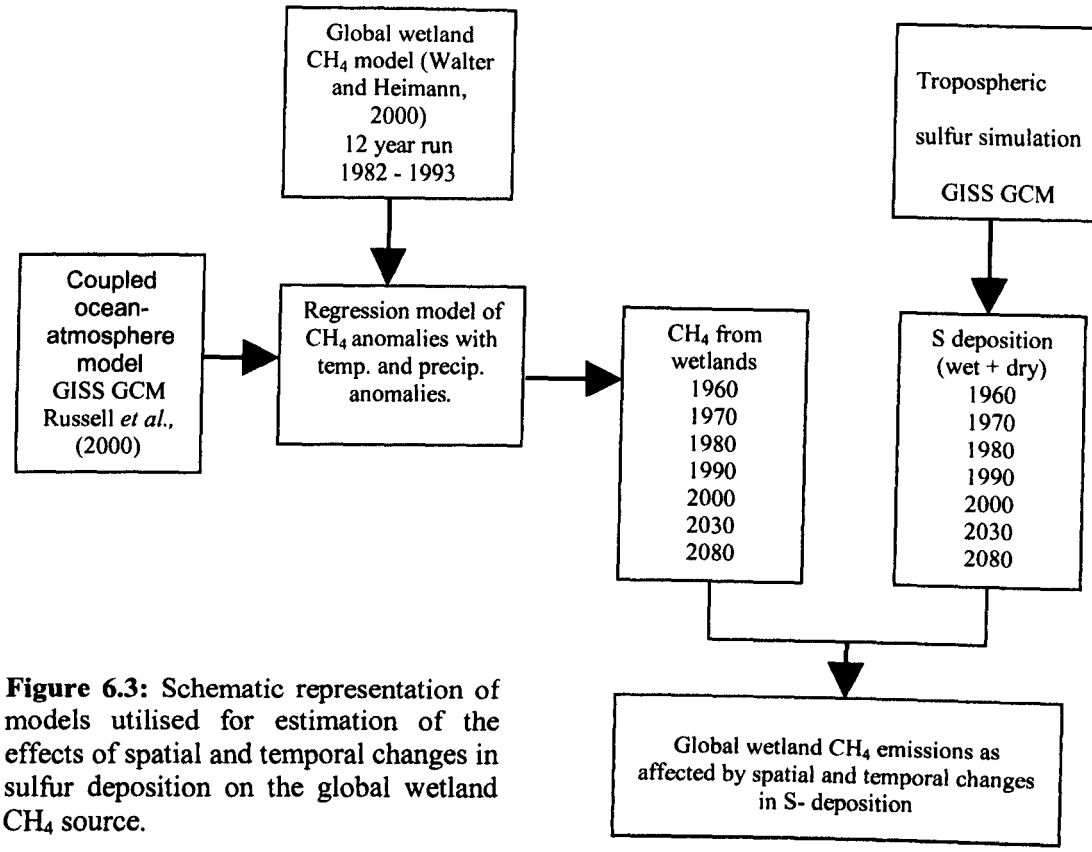

Figure 6.3: Schematic representation of models utilised for estimation of the effects of spatial and temporal changes in sulfur deposition on the global wetland CH_4 source.

estimated emissions data compiled for the Special Report on Emissions Scenarios (IPCC, 2000). For a full description of the model see Koch *et al* (1999). Modelled sulfur deposition output was produced on the standard GISS GCM 4° latitude x 5° longitude grid as the sum of wet and dry sulfur deposition. Throughout this Chapter, it is assumed that all S is deposited either in the SO_4^{2-} form, or, in the case of dry deposition, is oxidised to SO_4^{2-} when deposited on wetlands.

6.2.3 Combining S-deposition and CH_4 emission for global estimates of the "S-effect".

Both CH_4 emission and S deposition modelled output were combined for each year as summarised in the above schematic (fig. 6.3). Three estimated scenarios of the SO_4^{2-} deposition/ CH_4 emission interaction were used to combine the modelled output. Firstly, interaction scenario 'a' is the control where it is assumed that there is no

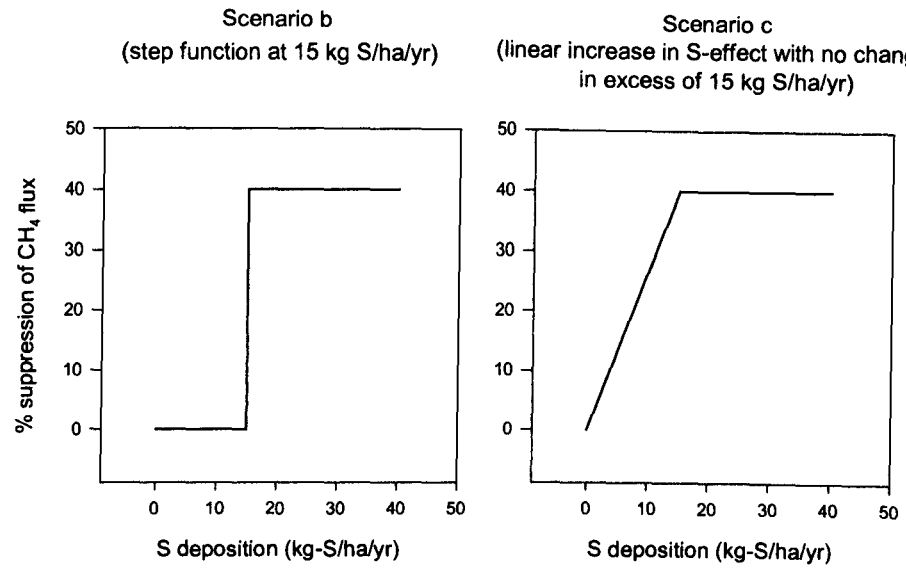

Figure 6.4: Visual description of the 'S-effect' treatment scenarios employed in this study i.e. a step function of 40% reduction in emissions with an excess of 15kg-S $ha^{-1}yr^{-1}$ (left), and a linear increase in 'S-effect' to 40% reduction at 15kg-S $ha^{-1}yr^{-1}$, a rate above which there is no further suppression (right).

interaction. The second (scenario b, fig 6.4) assumes that rates of SO_4^{2-} deposition below 15 kg-S $ha^{-1}yr^{-1}$ do not affect natural wetland CH_4 emissions. At rates of SO_4^{2-} deposition in excess of 15 kg S $ha^{-1}yr^{-1}$ a 40 % suppression of CH_4 emissions is assumed, regardless of the SO_4^{2-} input. This interaction scenario is based on observations reported by numerous workers (Dise and Verry 2001; Fowler et al., 1995; Granberg *et al.*, in press) for peatlands in N. America, Scotland and Sweden respectively as well as observations reported in this thesis (Chapters 3 and 5). The assumed lack of significant effect beneath the assigned threshold is based on the fact that there is a gap in understanding of the S deposition /CH_4 suppression link at these low rates of SO_4^{2-} deposition through a lack of experimental evidence. Interaction scenario 'c' is an alternative scenario in which there is a linear relationship between SO_4^{2-} deposition at deposition rates of less than 15 kg S $ha^{-1}yr^{-1}$. This scenario reflects the results of an investigation of SO_4^{2-} reducing activity of peat soils from peatlands spanning a global S deposition gradient (Vile *pers. comm.*), where SO_4^{2-} reduction rates

were found to increase, almost linearly, with increases in S deposition before plateauing at rates of S deposition in excess of 15-20 kg S $ha^{-1}yr^{-1}$. While it is recognised this does not show how CH_4 emissions may have been affected at low rates of S deposition, enhanced SO_4^{2-} reducing activity implies a larger population of competitively superior sulfate reducing bacteria (SRB) that may limit methanogenesis (Chapter 4). As such, I consider interaction scenario 'c' to be the best current approximation of how different rates of S deposition may affect CH_4 emissions.

6.3 Results and Discussion

6.3.1 Sulfur deposition

Global annual SO_4^{2-} deposition rates generated from simulation runs for 1960, 1990 and 2030 are presented in figure 6.5 to show the regional changes in SO_4^{2-} deposition during the 70 year period. In Europe, there is an increase in S deposition between 1960 and 1990 followed by a decline in 2030 as the anticipated abatement strategies take effect (fig 6.5 a-c). This is reflected in the declining area in North America and Europe exposed to S deposition >15 kg-S $ha^{-1}yr^{-1}$ (fig 6.5 i-iii). However, Eastern Europe remains a significant source of S pollution in 2030, and, because much of this S emission is expected to be deposited in the region, Eastern Europe remains a zone of high SO_4^{2-} deposition.

Under scenario 'b' (fig 6iii), a far larger area would still receive S deposition in excess of 8 kg $ha^{-1}yr^{-1}$ (fig. 6.5 c). The northeast United States shows little difference in deposition between 1960 and 1990 because despite efforts to reduce acid rain, emissions

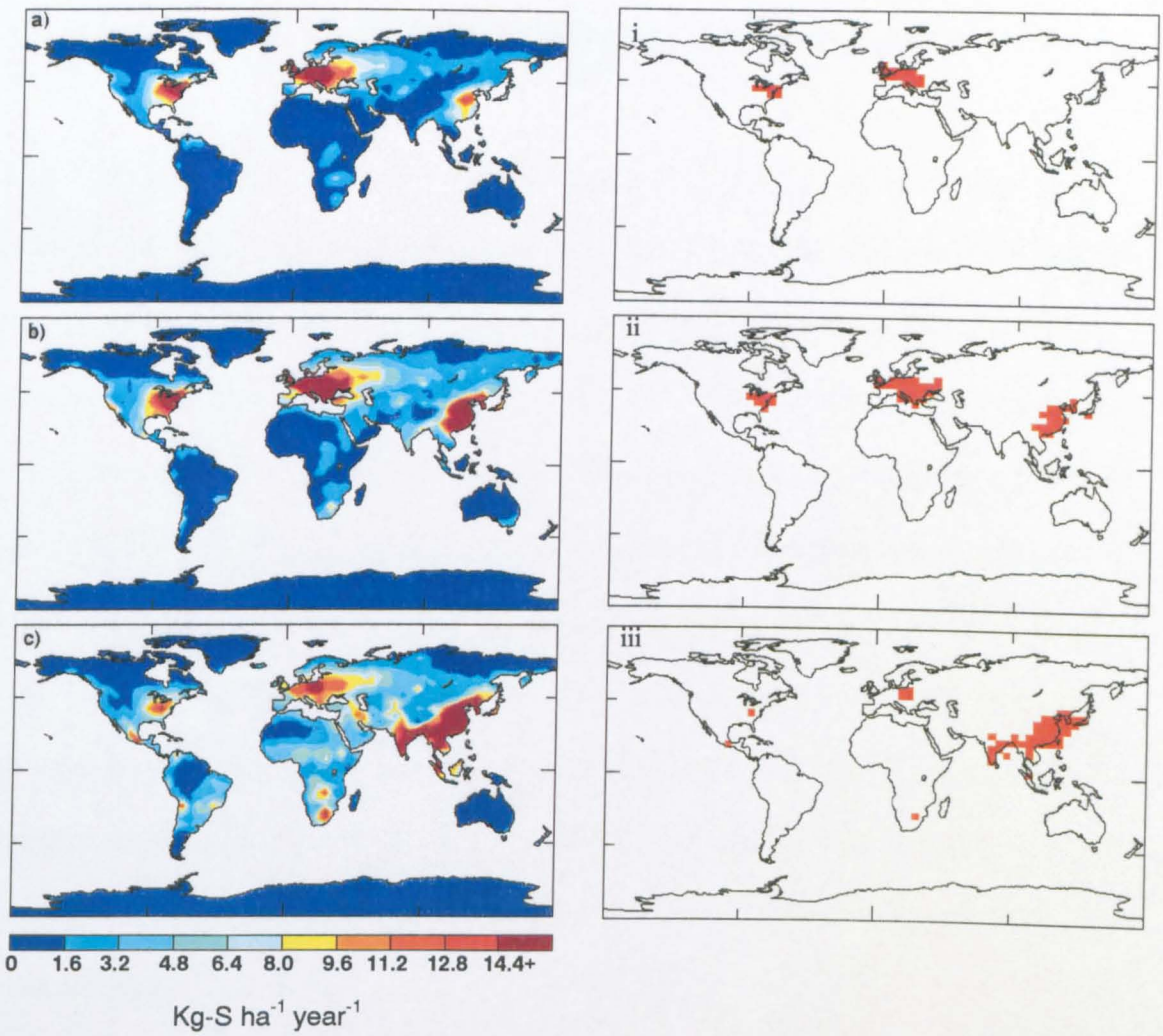

Figure 6.5: Global interpolated distribution of total (wet + dry) S-deposition (kg ha^{-1} $year^{-1}$) for the years 1960 (a), 1990 (b) and 2030 (C). The colour scale has been artificially shortened to show regions impacted by S-deposition at rates up to 15 kg ha^{-1} yr^{-1}. Areas impacted with S in excess of the 15 kg ha^{-1} yr^{-1} for the same years are shown in the panels on the right (i,ii,iii respectively).

of S changed little in the region over that time period. However, the anticipated decrease in deposition through to the year 2030 is dramatic.

In Asia, there is a continuous growth in the modelled area receiving S deposition through the 20th century until, in 2030, the region becomes the most impacted by S deposition globally. Comparison of the different S-treatment scenario impacted areas (6.5 c and iii) shows that the majority of the region is predicted to receive rates of S deposition in excess of 15 kg ha^{-1} yr^{-1}.

6.3.2 Temporal changes in the wetland CH_4 source strength due to climate change and the effect of S deposition on the global wetland source.

Global estimates of CH_4 emission from wetlands as derived from the adaptation of the wetland CH_4 emission model are around 250Tg for 1960 as described in more detail by Walter (1998). This is far larger than the latest estimate of the preindustrial wetland CH_4 source strength of around 160 Tg, which was derived from a three-dimensional chemistry-transport model in combination with isotopic analysis of CH_4 trapped in polar ice cores (Houweling *et al.*, 2000). The reasons for this overestimation in the model are not clear. Increases in mean annual temperatures between 1860 and 1960 were no more than 0.3 degrees (fig 6.2). Sensitivity tests of the model show that to gain a 20% increase in CH_4 emissions through temperature increases alone a 1°C temperature increase is required (Walter 1998). The small temperature increase during this time period is therefore, unlikely to explain such a large discrepancy (90 Tg ≡ 56% increase in emissions).

A reason for the large discrepancy may be that validating data sets used to parameterise the model were a) biased towards high CH_4 emitting wetland sites or b) derived from locations experiencing little pollutant S input. Indeed one of the assumptions of the model is that alternative electron acceptors such as SO_4^{2-} are depleted (Walter 1998). In addition to ombrotrophic wetlands that receive inputs of S solely via atmospheric deposition, other wetlands may overly sediments of oceanic origin from which they can substantial inputs of S may be derived (Rejmankova and Post 1996; Reeve *et al.*, 1996). This could potentially reduce both CH_4 production and emission. Many wetland regions of the world may fall into this category including the Hudson Bay Lowland (HBL), the second largest area of peatland in the world after the Western Siberian Lowland, and a region where CH_4 emissions are unusually small (Roulet *et al.*, 1994).

Reeve *et al.*, (1996) demonstrated that SO_4 concentrations in peat of the HBL were high for an area unaffected by S pollution, the likely cause of which is that the peat is underlain by SO_4^{2-} -rich oceanic sediments which were deposited during rapid sea level rise associated with termination of the last glacial period (Reeve *et al.*, 1996).

To provide a better representation of the global wetland CH_4 source and to achieve a more realistic representation of the most likely effects of atmospherically deposited S, modelled global CH_4 from 1960 was scaled down so that the estimated annual output of CH_4 was equivalent to the estimated pre-industrial source strength (Houweling *et al.*, 2000). The scaling factor was then applied to modelled CH_4 emissions fields for each subsequent year of the study (i.e. 1970-2080).

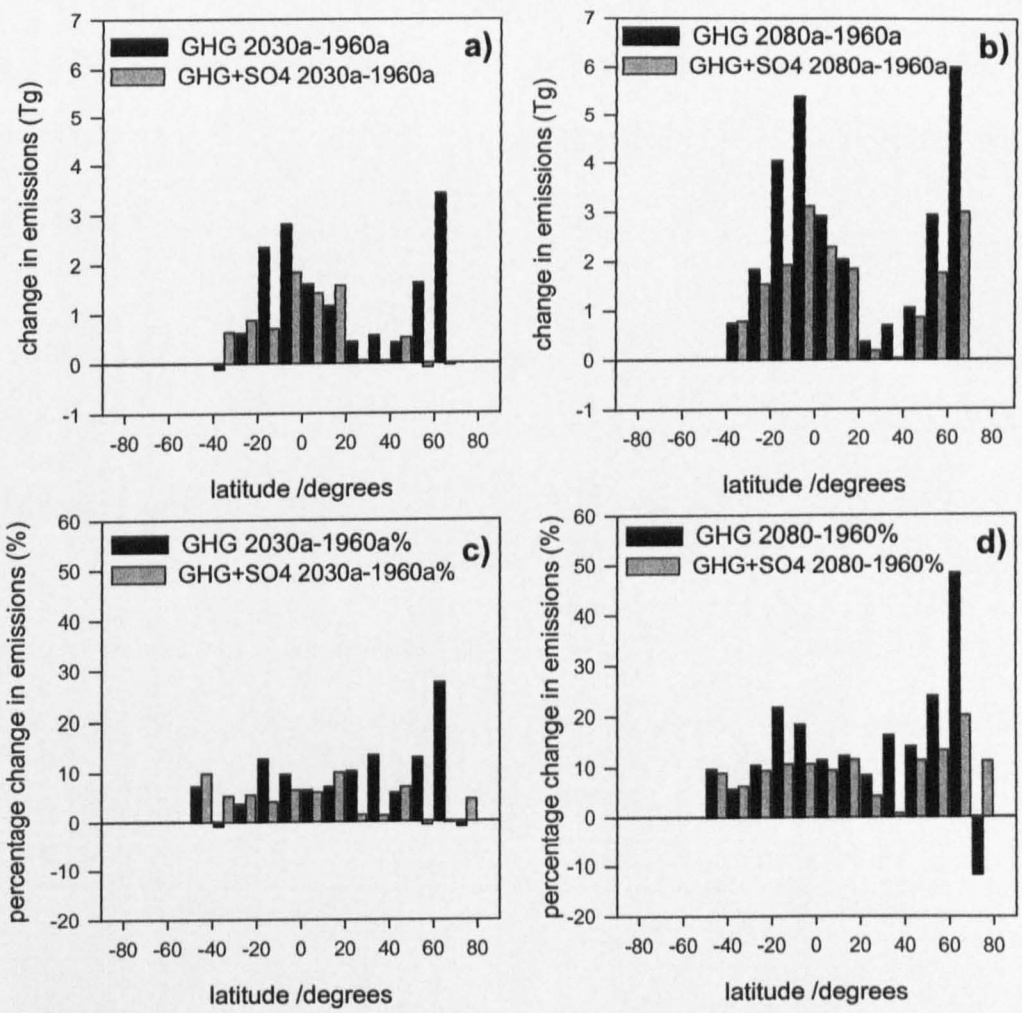

Figure 6.6: Mass (a and b) and percentage (c and d) latitudinal anomalies in CH_4 emission between 1960 and 2030 (a and c) and between 1960 and 2080 (b and d) for GHG and GHG+SO4 aerosol climate forcing scenario.

The results of the scaled down CH_4 model estimate predicts that the global CH_4 source will grow over much of the earth's surface under both GHG and GHG+ SO_4^{2-} climate-forcing scenarios (fig. 6.6). The largest proportional increase in emissions is predicted to take place in high latitude wetlands (60-70° latitude) (fig 6.6 c and d) as these areas are forecast to experience the largest temperature increases. The largest total mass increase, on the other hand, comes from low latitudes (fig. 6.6 a and b). Between 1960 and 2030, the percentage growth in CH_4 under the GHG+SO4 climate-forcing scenario is similar over the latitudinal range which is likely due to aerosols negating much of the warming that is predicted in high latitudes over this time period (fig 6.6 c light bar) (Russell et al., 2000). As the aerosol burden decreases in the mid to late 21st century, this damping effect is relieved and the proportional increase in 2080 emissions relative to 1960 emission is largest in the high latitudes (fig 6.6 d). Although a 10% decrease in emissions is predicted between 1960 and 2080 at 70-80° latitude under the GHG scenario, this is of negligible importance globally as this latitude is an insignificant CH_4 source (fig 6.6 b and d).

By incorporating the two different "S-effect" treatment interactions and through analysis at more frequent time intervals, a clearer picture of the effects of both climate change and S deposition emerges (fig 6.7). While the wetland CH_4 source increases from 1960 to 2080 due to the effects of both climate change scenarios (i.e. GHG only and GHG+SO4, 1 and 2 respectively, figs 6.7 i and ii – solid line) the rate of increase varies substantially under the GHG+SO4 climate scenario while progressing almost linearly under the GHG only scenario. In general, total CH_4 emissions, without S-deposition effects, are predicted to grow to around 190 Tg and 183 Tg under the GHG and GHG+SO4 climate-forcing scenarios respectively, from an initial source of little over 160 Tg.

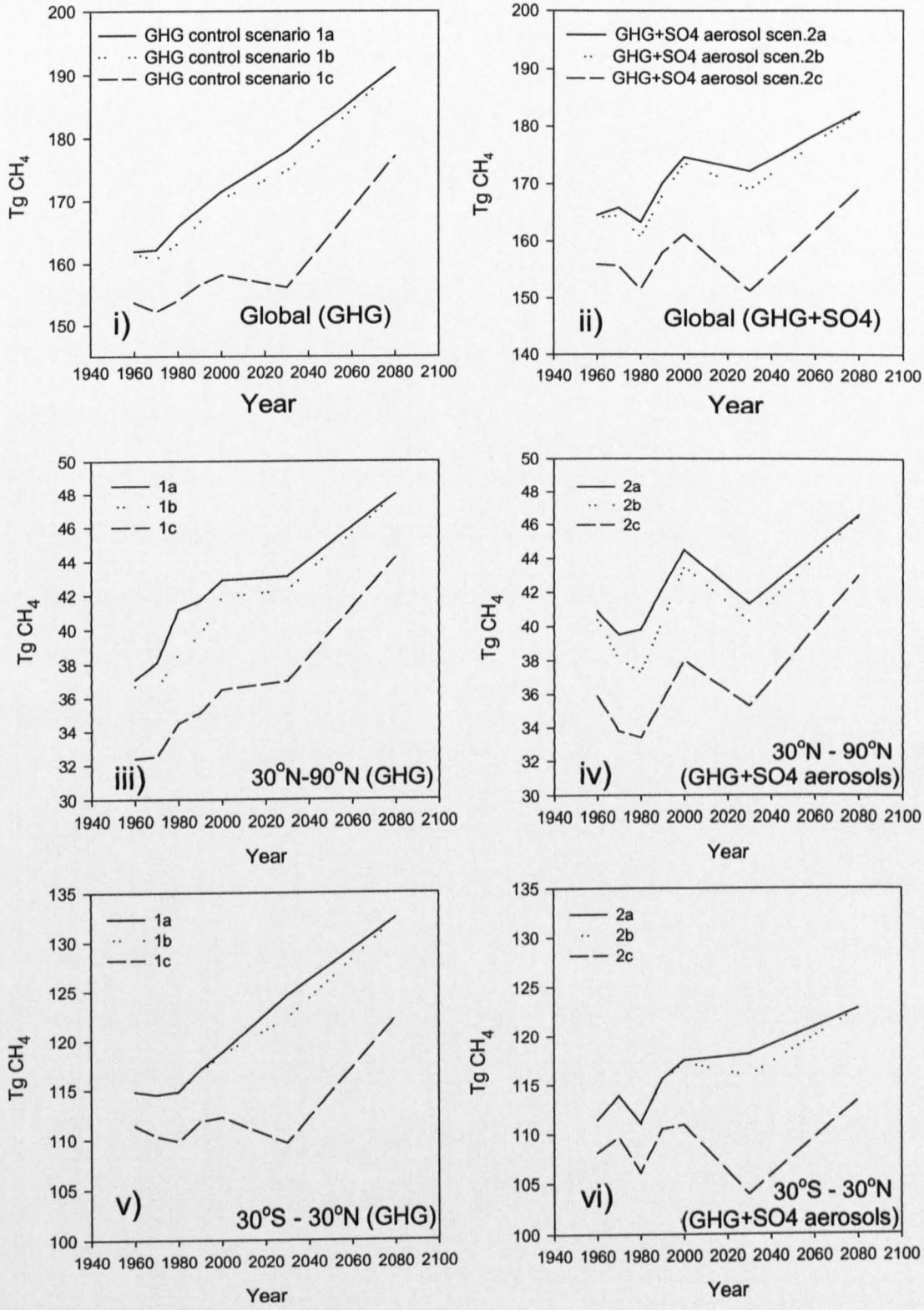

Figure 6.7: Estimated and projected changes in CH_4 emissions from the wetlands under different climate change scenarios (1 = GHG only; 2 = GHG + SO_4^{2-} aerosols) and three different "S-deposition effect" scenarios (a = no change, control; b = step decrease in CH_4 flux by 40% with S-deposition in excess of 15 Kg-S $ha^{-1}yr^{-1}$; c = as for b except a linear decrease in flux with increase in S-deposition up to 15 Kg-S $ha^{-1}yr^{-1}$.). Results are presented for the global wetland CH_4 source (i and ii), mid to high northern latitudes (iii and iv) and low latitudes (v and vi).

Mid to high latitudes exhibit a rapid increase in CH_4 source strength between 1960 and 1980, under the GHG scenario, although there is a decrease in the rate of increase between 1980 and 2030, a profile which closely follows the measured decline in the atmospheric CH_4 growth rate during this period (Dlugokencky *et al.*, 1998) before stabilising between 2000 and 2030 (fig 6.7 iii).

Treatment interaction scenario b, (step function in CH_4 response in excess of 15kg S ha^{-1} yr^{-1}) shows little effect on the source strength of mid to high latitude wetlands, as for other climate change scenarios and latitudes (fig. 6.7 i-iv, dotted line). Treatment interaction c, however, consistently reduces annual emissions by around 6-7 Tg (or by around 16%) between 1960 and 2030 while maintaining the same growth rate profile (fig. 6.7 v-vi, dashed line). For all latitudes, beyond 2030, however, the suppressive effect of this treatment scenario decreases, as S deposition is predicted to decrease during that time (fig 6.7).

The wetland source from low latitudes under this climate change scenario shows a very different profile with zero change in source strength between 1960 and 1980 before subsequently increasing almost linearly with time (fig 6.7 v and vi). The effect of treatment interaction c is large within this latitudinal range, maintaining an approximate zero growth rate between 1960 and 2030 with an anticipated difference in CH_4 emissions between control and S treatment scenario c of around 14Tg (or around 12 %) by 2030.

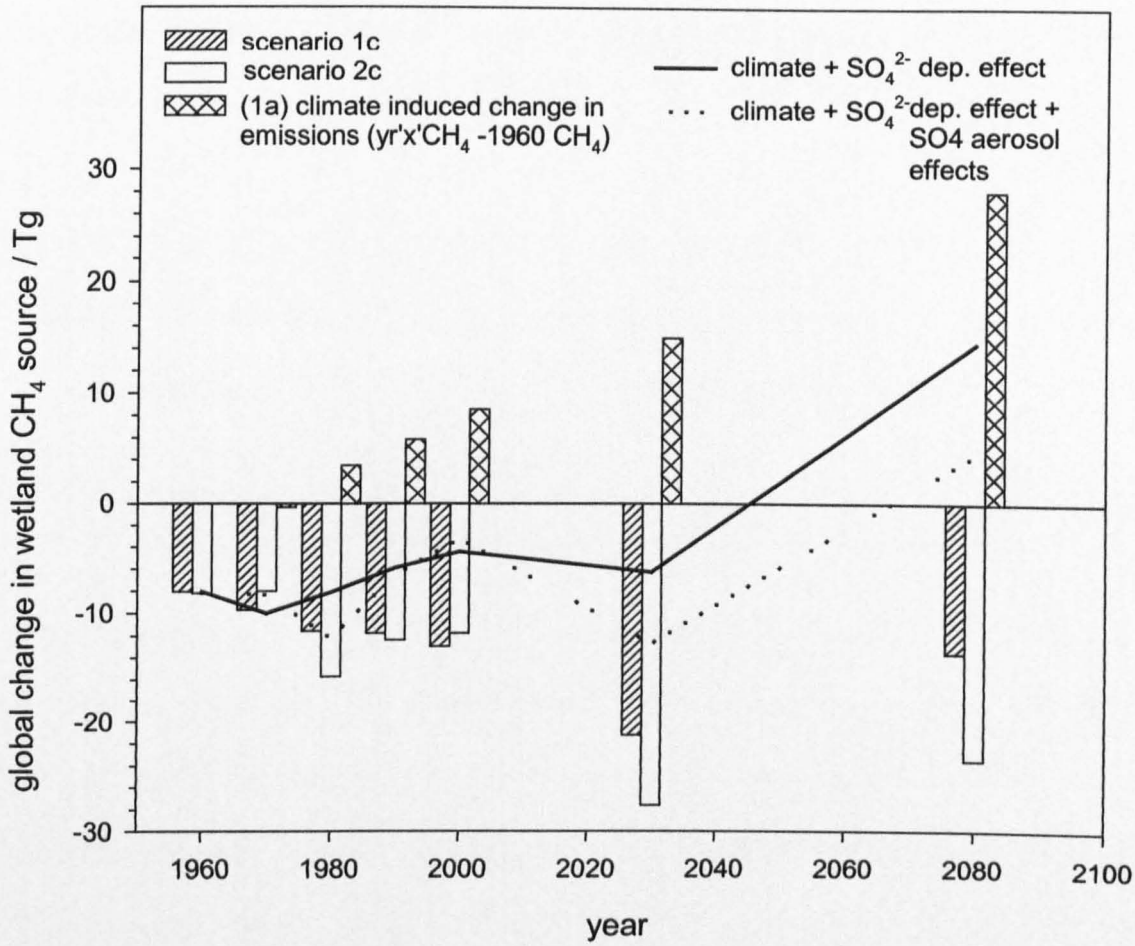

Figure 6.8: Estimated global change in CH_4 emission brought about through projected changes in climate (Russell *et al.*, 2000). The estimated negative impact of SO_4^{2-} deposition on CH_4 emissions is shown (negative y axis) for scenarios 1c (i.e. linear treatment response of up to 15 kg S-deposition on the control (GHG only) climate forced wetland emission scenario) and 2c (same treatment response but with GHG+SO_4^{2-} aerosol climate change scenario (Russell *et al.*, 2000)). The solid line indicates the net estimated effect of climate change and S deposition on the wetland CH_4 source. The dotted line indicates the estimated combined effect of S deposition, projected climate change and climate changes associated with SO_4^{2-} aerosols on global CH_4 emissions.

In the GHG+SO4 aerosol climate scenario there is a general increase in the wetland source strength although there is a pronounced decrease in global CH_4 emissions under any S deposition scenario during two time periods, (1970 and 2000 (fig 6.7 ii)). This is most likely due to increased SO_4^{2-} aerosols during these two time periods (high northern latitudes in 1970-1980 and low latitudes in 2000-2030) increasing atmospheric albedo and therefore decreasing mean surface temperatures. The recovery in the growth of the wetland CH_4 source in the intervening time is most likely due to a reduction in the

atmospheric aerosol burden as a result of western pollution abatement policies, which precedes the predicted growth in pollutant S emissions from Asia.

The effect of S deposition on CH_4 emissions under the GHG+SO4 climate forcing scenario with treatment interaction c, is large enough in low latitude regions, for there to be an overall decline in the CH_4 source between 1960 and 2030 (fig 6.7.vi).

6.3.3 Net effect of both climate change and S-deposition on the wetland CH_4 source

The estimated combined effects of climate change, aerosols and S-deposition on CH_4 emissions over time and is presented in figure 6.8. These findings show that future S deposition may be sufficient not only to offset the predicted growth in CH_4 emissions, from enhanced greenhouse warming, but also to reduce the global wetland source to below that of preindustrial times by approximately 8 Tg during the second half of the late 20^{th} century (fig 6.8). Predicted increases in the production and deposition of oxidised S compounds through economic growth over the next 30 years are likely to suppress emissions even further. A predicted annual "S-effect" suppression of 22 Tg more than offsets the predicted GHG induced increases in annual CH_4 emissions of 15 Tg over this period. Eventually, predicted growth in CH_4 emissions due to climate change outweigh the suppressive effect of S-deposition and the wetland CH_4 source becomes larger than the estimated preindustrial source strength, sometime between 2030 and 2080. The combined effect of SO_4^{2-} aerosols (cooling effect) and SO_4^{2-} deposition are predicted to offset GHG warming induced growth in the CH_4 source by 28 Tg in 2030 and by 24 Tg in 2080.

The potential size of this suppression places the S-deposition/CH_4 flux suppression interaction in a similar size category to many other components of the CH_4 budget that

have received far greater attention. These components (together with their estimated size range) include the soil sink (15-45 Tg), the termite source (10-50 Tg), oceanic emission (5-50 Tg), animal waste (20-30 Tg), biomass burning (20-80 Tg) and landfills (20-70 Tg) (IPCC 1995). In addition the global rice source, which has received much attention, is now estimated to be far smaller than was previously thought (~30 Tg, Dernier van der Gon pers. comm.).

Finally, it is clear that the effects of SO_4^{2-} deposition on global CH_4 emission from wetlands, is critically dependent on the effect of low dose rates of SO_4^{2-} deposition (<15 kg-S $ha^{-1}yr^{-1}$). Future research should aim to improve understanding of interactions between SO_4^{2-} deposition and CH_4 emissions from wetlands at such low rates of SO_4^{2-} deposition

6.3.4 Uncertainties and study limitations

While the implications of this study are important for improved understanding of factors which may control changes in the largest CH_4 source, there are a number of simplifications and uncertainties which should be addressed.

Firstly the results reported here are global extrapolations of results from only a limited number of SO_4^{2-} manipulation experiments in peatlands (Chapters 3 and 5, Dise and Verry in press). There is a need, therefore, for similar experiments to be replicated for a range of wetland types. Secondly, and as already mentioned, the linear increase approximation in "S-effect" at the S-deposition range of 0 to 15 kg S $ha^{-1}year^{-1}$ is based on limited data on changes in SO_4^{2-} reduction rates within this range (Vile *pers. comm.*). Long-term low dose experiments, as are reported in chapters 3 and 5, but with experimentally enhanced S deposition rates within this range, should provide evidence

to enable global estimates of the suppressive S-effect, as reported here, to be estimated with greater confidence.

In addition I assume that the interaction between S-deposition and CH_4 flux is uniform regardless of latitude. It is shown in chapter 3 that although CH_4 fluxes are suppressed by around 40% annually, changes in temperature (as cooler temperatures stimulate a larger "S-effect" response; Chapters 3 and 5) and water table are important variables in determining the total suppressive effect of applied SO_4^{2-} treatments at a given time. Higher temperatures in low latitudes may therefore decrease the response of wetland microbial communities to enhanced SO_4^{2-}supply. Measurements of CH_4 emissions from tropical wetlands overlying sediments of varying SO_4^{2-} status do, however, indicate that the presence or absence of SO_4^{2-} is an important factor in determining CH_4 fluxes from these systems (Rejmankova and Post 1996). More studies similar to that reported in chapter 3, but in low latitude wetland systems would clarify the response of tropical wetlands to enhanced S supply through acid rain.

The period of recovery in CH_4 fluxes once S deposition is relieved, and the factors governing recovery are not understood. It is known that peatlands are net sinks of S and that S undergoes reduction and oxidation over short to long time scales, moving between pools of varying biological availability (Wieder et al., 1987; Freeman et al., 1994).

The approach reported here assumes that there is an immediate return to normal, pre-SO_4^{2-} rates of CH_4 emission. It is possible, however, that recovery of CH_4 flux on decreasing the S deposition load, may occur over even longer time scales, possibly

decadal, in which case the total suppressive "S effect" reported here should be considered an underestimate.

Finally as already discussed in this study, no account is made of wetland areas that may also receive S inputs from underlying geology that may be of oceanic origin. This is an important uncertainty that may account for a considerable proportion of the difference between the estimated size of the wetland source (~160 Tg - Houweling *et al* 2000) and the unadjusted modelled 'alternate acceptor free' estimate of wetland emissions (~250 Tg for 1960, Walter 1998). As already discussed, one of the largest boreal wetland regions in the world, the Hudson Bay Lowland (HBL), is known to overly SO_4^{2-} rich sediments (Reeve *et al.*, 1996). Efforts should therefore be made to establish global data sets of areas where wetlands and SO_4^{2-} rich geology coincide.

6.4 Conclusions

This study suggests that S deposition from acid rain could not only potentially negate the effects of observed and predicted climate warming on the global natural wetland CH_4 source, but may also potentially suppress the wetland CH_4 source to a level below that which was estimated during preindustrial times. The suppressive effect of S deposition may be as important to the CH_4 budget as other components of the budget that have received far greater attention.

The work presented in this Chapter is the first attempt at examining this interaction at the global scale. As such, there are considerable uncertainties and limitations, which may be reduced through further research of the "S-effect" interaction, particularly for a

broader range of wetland types and from a larger climatic range than has already been examined.

This suppressive effect may decrease sometime in the mid 21st century as pollution controls and cleaner technologies take effect globally, thereby causing the wetland CH_4 source to increase beyond the natural preindustrial source strength in response to climate warming. The rate of recovery of previously S impacted wetland CH_4 emissions in response to a decrease in the S deposition load is a considerable uncertainty.

Chapter Seven

General Discussion.

7.1 Introduction

Chapters 2-6 detail a variety of approaches that were employed in order to examine the questions outlined in Chapter 1. The aims of the study were to i) investigate the relationship between SO_4^{2-} deposition and CH_4 emissions within a range of SO_4^{2-} deposition commonly experienced in regions affected through acid rain, ii) to examine the role of environmental variables (i.e. temperature and water table) as a control on the effects of SO_4^{2-} on CH_4 emissions and iii) to examine the long-term consequences of SO_4^{2-} deposition on CH_4 emissions from peatlands

In addition to these investigations, made under both natural (Chapter 3) and manipulated controlled environment (Chapter 5) conditions, studies were undertaken on processes operating at the microbial scale. In particular, rates of SO_4^{2-} reduction and CH_4 oxidation as affected through changes in SO_4^{2-} deposition were studied (Chapter 4). Finally, the implications of the findings were extrapolated to the global scale to estimate the current and future potential global significance of the findings (Chapter 6).

In this Chapter, the work is discussed and summarised and recommendations for future investigations are made.

7.2 The effect of different rates of S deposition on CH4 fluxes.

In Chapters 3 (field experiment) and 5 (controlled environment experiment), two similar ranges of enhanced SO_4^{2-} -S deposition were applied. In combination, the rates of S deposition (applied as frequent small doses) were 15, 25, 50, and 100 kg S $ha^{-1}yr^{-1}$. In Chapter 5, the range of experimentally applied SO_4^{2-} included a single large application of SO_4^{2-}.

Both experiments yielded similar results. The Moidach More field experiment showed that prolonged additions of 25 kg SO_4^{2-} -S ha^{-1} yr^{-1}, a commonly experienced rate of S deposition in Europe, suppressed CH_4 emissions in the second year of the experiment by 36%. This suppression was statistically indistinguishable from the effect of much larger rates of SO_4^{2-} deposition e.g. 42% suppression in total CH_4 flux from 100 kg SO_4^{2-} -S ha^{-1} yr^{-1} applied in the same manner.

Peat samples from the experimental field site treated with 50 kg SO_4^{2-} -S ha^{-1} yr^{-1} exhibited significantly enhanced SO_4^{2-} reducing potential and trends of reduced CH_4 oxidation potential (Table 4.1). This suggests that the growth of an enlarged population of SRB (table 4.1), which competitively excludes methanogens from access to substrates, is the likely cause of the long-term suppression and that decreasing methane production limits the growth of low affinity methanotrophs (which have a high CH_4 threshold concentration (Oremland, 1988)) (Chapter 4). In addition, peat pore-water concentrations of CH_4, were around 50% smaller in plots treated with 50 kg-S $ha^{-1}yr^{-1}$ when compared with controls in the field (Chapter 3).

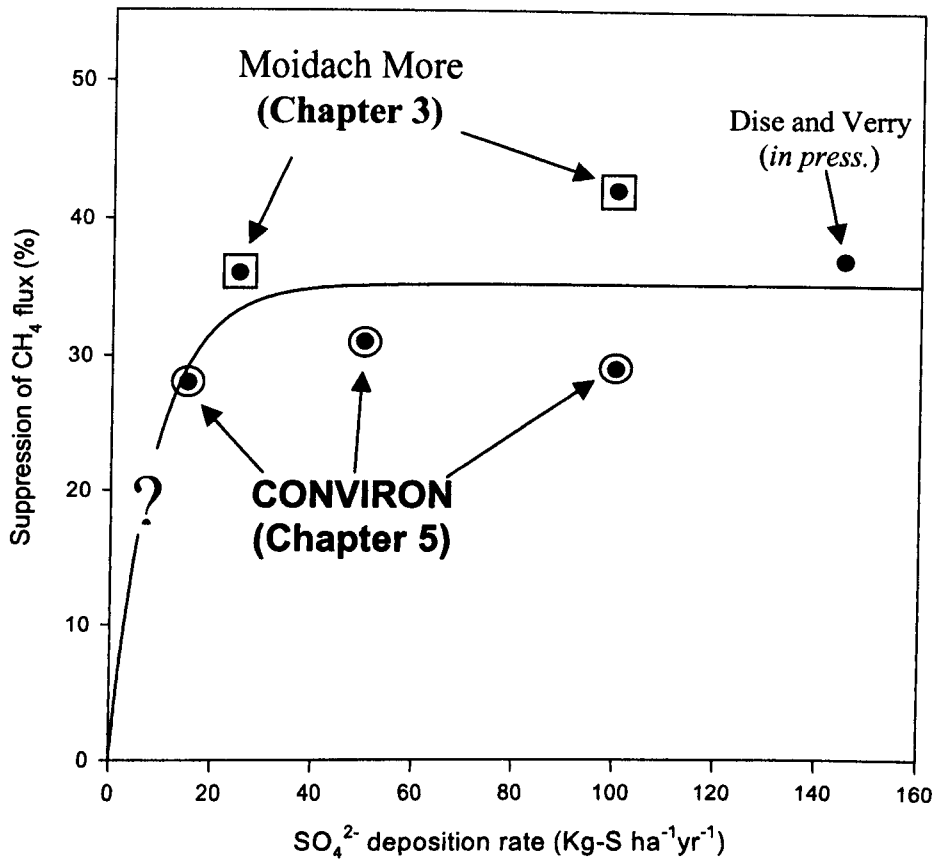

Figure 7.1: Summary of results of S manipulation experiments in peatlands (from this study and from literature). Each point represents the mean suppression over the length of the experiment. The 50 kg-S treatment result from Moidach More is omitted due to the large number of plants in these plots, which increased emissions and decreased the difference in fluxes relative to controls. Trend line represents an estimate of the response of CH_4 emissions to SO_4^{2-} deposition.

Although a similar degree of flux suppression was apparent during the first year of the experimental applications in Moidach More, the differences were not statistically significant. This is in contrast with the findings reported in Chapter 5, where a progressive reduction in CH_4 emissions from monoliths treated with low levels of SO_4^{2-} was observed relative to controls, soon after applications of SO_4^{2-} first started (fig. 5.4).

It is likely that the low water-table conditions that prevailed during the first year of the field manipulation, enabled the thick aerobic layer (generally the top 20cm of the peat

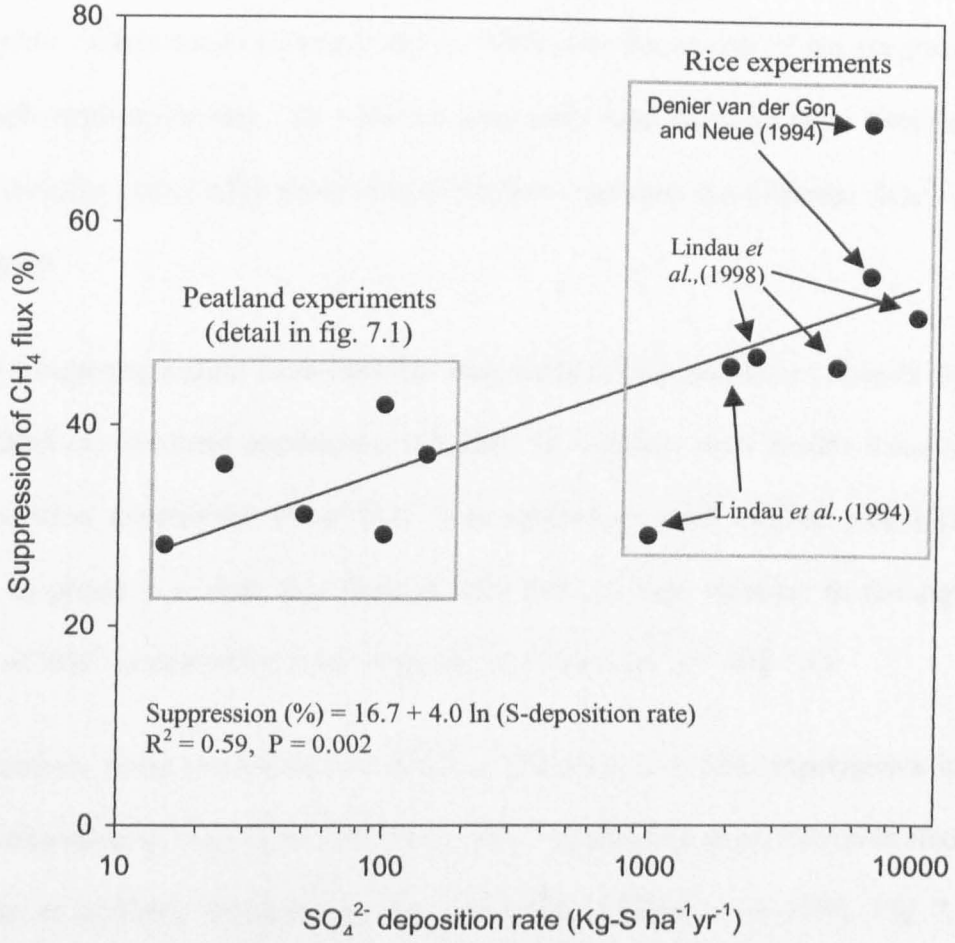

Figure 7.2: Combined results of enhanced SO_4^{2-} deposition experiments in both natural peatland systems and rice-based agro-ecosystems.

column during 1997 fig 3.2) to act as a buffer, reducing hydraulic conductivity and keeping separate the active methanogenic zone from SO_4^{2-} that had been experimentally applied. In the controlled environment experiment presented in Chapter 5, the water-table in each monolith was maintained at the peat surface and so any such buffer between a zone of maximum methanogenesis and aerial inputs of SO_4^{2-} was minimal in comparison.

In the CONVIRON study, total CH_4 flux suppression in SO_4^{2-} treated monoliths varied little between the different dose rates, which included a rate of 15 kg-S $ha^{-1}yr^{-1}$, the

lowest SO_4^{2-} deposition rate yet reported to exert a suppressive influence over CH_4 emissions. Suppression averaged around 30% over the length of the treatment period for each application rate. As with the long-term field experiment at Moidach More, there were no statistically significant differences between the different SO_4^{2-} treatment responses.

When comparing results from both the long-term field experiment (Chapter 3) and the controlled environment experiment (Chapter 5), together with results from one other manipulation experiment where SO_4^{2-} was applied as small regular pulses (Dise and Verry in press) it is clear that there is very little, if any, increase in the suppressive effect of SO_4^{2-} at deposition rates in excess of 15 kg S $ha^{-1}yr^{-1}$ (fig 7.1).

Furthermore, when comparing the results of frequent low dose experiments in natural peat wetlands with large dose fertilization SO_4^{2-} application experiments in rice paddies (Lindau *et al* 1994; Dernier van der Gon 1994; Lindau *et al* 1998. Fig 7.2), CH_4 emissions are suppressed by a similar percentage even if SO_4^{2-} applications are 3 orders of magnitude larger. In Chapter 5 it was shown that the addition of NaCl, at equivalent ionic concentration to the 50 kg-S $ha^{-1}yr^{-1}$ treatment, had no effect on CH_4 emissions. However, in far larger treatment applications, as would be the case in the rice experiments, ionic concentrations may be sufficient to adversely effect methanogens thereby adding to the suppressive effect of SO_4^{2-} in stimulating a functional change / community shift amongst microbial communities.

The overall suppressive effect of SO_4^{2-} additions was smaller in the CONVIRON monolith study (~30%) than during the second year of the Moidach More study (~40%). There are a number of reasons that may have caused this difference in response. One is the length of time peat was exposed to SO_4^{2-}. Moidach More had 6 months of addition

whereas peat in the CONVIRON experiment was exposed to SO_4^{2-} for only 2 months by the end of the experiment. In addition, background (control) SO_4^{2-} concentrations were different in peat from the two experiments; averaging around 33μM in Moidach More control plots and 64μM in CONVIRON control monoliths (figs 3.5 and 5.7). The larger concentrations of SO_4^{2-} in the peat monoliths collected from Caithness may reflect larger inputs of SO_4^{2-} to peat than was experienced in Moidach More in Morayshire, which may have resulted in enhanced competition with active sulfate reducing SRB favoured over methanogens. The 10% difference in suppression may support the hypothesis that at rates lower than 15 kg-S $ha^{-1}yr^{-1}$, SO_4^{2-} deposition may exert an influence over CH_4 emissions that increases with an increase in SO_4^{2-} deposition.

In general it is likely that SO_4^{2-} application rates in excess of 15 kg-S $ha^{-1}yr^{-1}$ are sufficient to stimulate a competitive population of sulfate reducing bacteria (SRB). At rates in excess of 15 kg S $ha^{-1}yr^{-1}$ other factors other than SO_4^{2-} supply may become limiting.

7.3 Temporal variability of "S-effect" in controlled environment studies.

All SO_4^{2-} treated monoliths in the CONVIRON study, regardless of treatment size or mode of treatment application (i.e. frequent small SO_4^{2-} pulses or a single large application) exhibited a pronounced period of CH_4 flux "recovery" around 4 weeks after the onset of suppression (Chapter 5). It was previously thought that single large applications of SO_4^{2-} would stimulate an SRB population "boom" followed by a "crash" as SO_4^{2-} is rapidly consumed from the peat system. It was hypothesised that small frequent applications would avert a "crash" by maintaining a constantly elevated SRB population (Arah and Stephen 1998). It was therefore surprising that CH_4 fluxes from monoliths affected by either mode of treatment application should respond in a similar

way with an apparent recovery. In light of the finding that CH_4 fluxes in treated plots in the long-term Moidach More field experiment (Chapter 3) were suppressed from the start of the second year of the treatment, and continued to be suppressed over the duration of the experiment, this finding is particularly enigmatic.

Two hypotheses were proposed in this thesis to account for this seemingly contradictory finding. The first assumes that during the later plant growth stages, labile carbon substrates are produced through a combination of enhanced production of root exudates followed by root degradation. This produces an excess of substrate which is capable of maintaining both SRB and methanogen populations (Chapter 5).

The second hypothesis is that changes in the SO_4^{2-} supply may temporarily affect microbial population dynamics before long-term, steady state conditions are established. Raskin *et al.*, (1996) found through the addition of SO_4^{2-} to a previously SO_4^{2-} free, methanogenic fixed-bed biofilm reactor that was inoculated with natural fresh-water microbial communities, that SRB populations first increased and then decreased over a 50 day period before increasing again to an elevated steady-state population after 100 days. However no explanation for this initial variability is offered. The time scale over which the initial changes occurred (50 days) is similar to that of the changes observed in the CONVIRON monoliths.

In addition, there is the possibility that initial suppression of CH_4 emissions following elevated SO_4^{2-} inputs, initially reflects a "de-coupling" of syntrophic SRB/ methanogen consortia where previously, SRB provided H_2 substrates for methanogens through "inter-species hydrogen transfer" (Conrad *et al.*, 1987) rather than the generation of an enlarged and competitively superior population of SRB.

This disassociation would deprive methanogens of a primary substrate, H_2, and so CH_4 production would decrease as SRB instead produce H_2S (Conrad *et al.*, 1987). An interesting speculation is that the recovery in CH_4 emissions may possibly be the result of methanogens, which were previously associated with SRB, forming new mutually beneficial associations with obligate syntrophic bacteria (e.g. *Syntrophomonas*, McInerney *et al.*, 1981) in lieu of SRB as H_2 providers, which have yet to form an enlarged competitive population. It can take considerable lengths of time (100+ days) for SRB populations to grow when presented with an enhanced SO_4^{2-} supply (McCartney and Oleszkiewics 1993; Raskin *et al.*, 1996). In the long-term (100+ days) populations of SRB may become sufficiently numerous to competitively exclude methanogens over available substrates. These are likely to include H_2 that is being transferred, inter-specifically, to methanogens (Abram and Nedwell, 1978) since this is the most probable method with which methanogens access H_2 (Conrad *et al,* 1985; McCartney and Oleszkiewics 1993; Raskin *et al.*, 1996). Indeed, on the three occasions where sulfate reduction potential was measured at Moidach More (after 4, 14 and 18 months of enhanced SO_4^{2-} additions) significantly larger sulfate-reduction potentials were only measured during the last sampling period, 18 months after experimental additions began (figure 4.1). A hypothetical conceptual model of how microbial communities may respond to enhanced SO_4^{2-} supply over both the short and long-term, is presented in figure 7.3.

An implication of this is that the short-term controlled environment studies (over 2–3 months) as reported in Chapter 5 may be limited to a period where microbial communities are restructuring in response to a SO_4^{2-} perturbation. Longer-term controlled environment studies are required in order to test this hypothesis. Furthermore, detailed SO_4^{2-} field manipulation experiments, where individual microbial

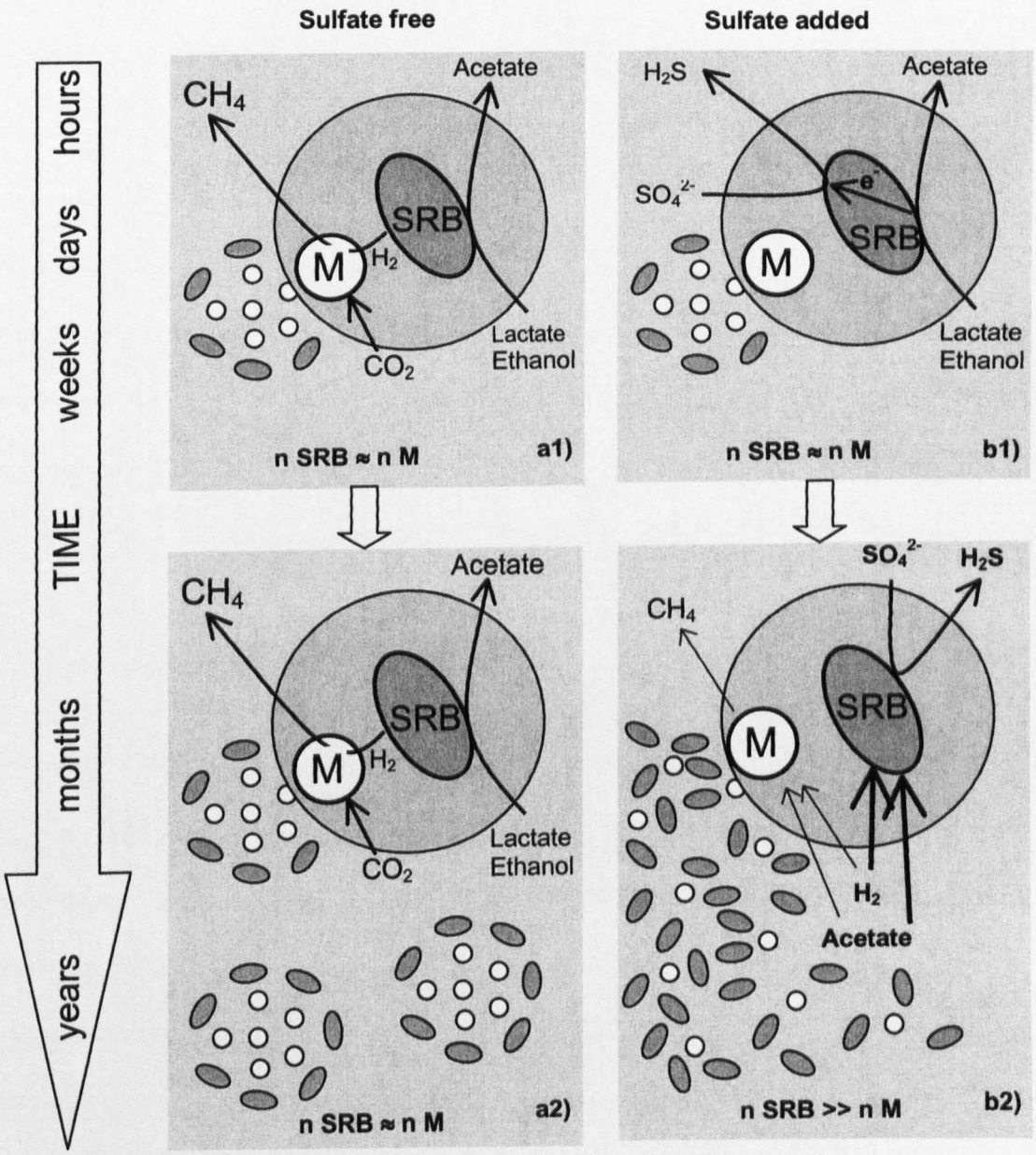

Figure 7.3: Hypothetical conceptual model of changes in SRB metabolism and microbial community structure with time. 'M' and 'SRB' represent, respectively, generic methanogens (white circles) and generic SO_4^{2-} reducing bacteria (grey ovals). Panels a1) and b1) are after Conrad 1989. The shift in activity depicted from panel b1) to b2) represents growth of a competitively superior SRB population with time. Thickness of arrows in panel b2) represent the relative size of pathways of substrates and SO_4^{2-}.

populations and substrates are monitored, in addition to net CH_4 emission, would enable the evaluation of these proposed hypotheses (fig. 7.3).

7.4 Variables controlling the extent of CH_4 flux suppression by enhanced SO_4^{2-} deposition.

The long-term field SO_4^{2-} manipulation experiment and controlled environment experiment both facilitated the examination of variables that may influence the extent of CH_4 flux suppression through SO_4^{2-} deposition by acid rain. Monitoring CH_4 fluxes, peat temperature and water table over 20 months of the field based experimental manipulation allowed natural, seasonal changes in these variables to be examined in relation to each other. Under controlled environment conditions, peat temperature was manipulated (at 5, 10, 15 and 20°C); thereby allowing CH_4 emissions from both SO_4^{2-} treated and control monoliths to be examined in response to applied temperature constraints.

7.4.1 Temperature effects

It is clear from the data presented in both Chapters 3 and 5 that not only are peatland CH_4 emissions affected by changes in temperature but that the extent to which SO_4^{2-} suppresses CH_4 flux may also be temperature dependent. In the field experiment reported in Chapter 3, there is a strong increase in the suppressive effect of SO_4^{2-} deposition with a decrease in temperature and also a decrease in water table (Fig. 3.6; $R^2 = 0.56$, $P < 0.0001$ for combined effect; see section 7.4.2 for a detailed discussion of the effects of water table).

Similar findings were made under manipulated temperature conditions in the controlled environment study detailed in Chapter 5. CH_4 fluxes responded less to increases in temperature, within the range of 5-15°C, in peat monoliths treated with larger SO_4^{2-} application rates (Q_{10} of 1, i.e. no change in the 100 kg-S $ha^{-1}yr^{-1}$) than did control monoliths (Q_{10} of around 2) (fig 5.7). In addition, within a higher temperature range (15-20°C), while control monoliths responded with a similar Q_{10} (2-2.5), CH_4 fluxes from monoliths treated with SO_4^{2-} responded with a far higher Q_{10} than was measured within the lower temperature range (Q_{10} of 4) (fig 5.7). This increase in CH_4 flux response in treated monoliths at higher temperatures is interpreted to be the result of a recovery in emissions from a more 'SO_4^{2-} suppressed' state at cooler temperatures to less suppression at higher temperatures.

In Chapter 3 it is hypothesised that temperature may affect competition between SRB and methanogens as the two microbial groups have different uptake rates for H_2 and acetate substrates (Kristjansson *et al.*, 1982; Schonheit *et al.*, 1982; Bodegom and Stams 1999). Working with rice paddy soils, Bodegom and Stams (1999) found that competition over H_2 favours SRB exclusively. In contrast, uptake rates for acetate are much closer in SRB and methanogens and so temperature may affect competition with methanogenesis favoured at higher temperatures and SRB favoured during cooler periods (Bodegom and Stams 1999). Since the methanogenic pathway in peatlands is known to shift from a predominance of CO_2 reduction (H_2 substrate) during the winter, to aceticlastic methanogenesis in the summer, the temperature effect on SO_4^{2-} suppression of CH_4 fluxes may reflect this (Kelly *et al.*, 1992; Avery *et al.*, 1999).

7.4.2 Effects of water table changes on CH_4 flux suppression.

In addition to temperature influences, enhanced periods of CH_4 flux suppression in SO_4^{2-} treated plots were also observed during the Moidach More experiment during times when the water table was temporarily lower than was usual for the year (1998 Chapter 3). Two hypotheses to explain this finding are proposed. The first is based on the finding that significantly less CH_4 was found dissolved in pore water extracted from SO_4^{2-} treated plots than controls (fig 3.5). It is proposed that the fact that there is less CH_4 dissolved in treated pore water together with the low solubility of CH_4 means that there is less accumulated CH_4 to be released to the atmosphere relative to controls during periods of declining water table, thereby temporarily accentuating the effect of the SO_4^{2-} treatment.

Alternatively, or in addition to this mechanism, it is known that SO_4^{2-} concentrations increase in aerobic upper layers of the peat column as reduced S compounds (e.g. H_2S, the product of dissimilatory sulfate reduction) are reoxidised to SO_4^{2-} (Freeman *et al.*, 1994). A lower water table may increase the proportion of H_2S that is oxidised to SO_4^{2-} in treated plots and so this mechanism may provide a temporarily more enhanced supply of SO_4^{2-} than would be made available via this mechanism to controls.

7.5 S dynamics in experimentally manipulated peat.

An intriguing finding of both the long-term field experiment at Moidach More and the short-term manipulation experiment under controlled environment conditions is that, excluding the uppermost 5cm of the peat column (where, as expected, SO_4^{2-} concentrations were highest in treatments), dissolved SO_4^{2-} concentrations in pore water at various depths below the peat surface, are lower in SO_4^{2-} treated plots and monoliths

than in controls. This counter-intuitive finding, which was observed in Moidach More treatment plots exposed to months of elevated SO_4^{2-} deposition (figs. 3.5 and 4.2) as well as in SO_4^{2-} treated monoliths after only a short period of exposure to enhanced SO_4^{2-} loads (fig. 5.8) provokes re-examination of the view that *in situ* SO_4^{2-} concentrations determine the degree to which carbon flow is mediated by SO_4^{2-} reduction in freshwater systems (Lovley and Klug, 1986).

A possible explanation for this decrease in SO_4^{2-} concentrations in peat receiving enhanced SO_4^{2-} deposition is that the enhanced supply of SO_4^{2-} may stimulate the formation of an active 'SO_4^{2-} reducing' SRB population (from previously methanogenic SRB/methanogen syntrophic consortia) that may be able to scavenge available SO_4^{2-} to concentrations that are smaller in treatments than in controls (Chapters 3, 4 and 5).

Very little of the SO_4^{2-} that was applied to the peat monoliths was emitted from the peat surface as volatile reduced S compounds (<1%)(Chapter 5). This implies that the majority of SO_4^{2-} that is deposited via acid rain is retained by peatlands.

Different sulfur pools in the peat were examined at a single depth in monoliths receiving 50 kg-S $ha^{-1}yr^{-1}$ and controls. As analysis of the S pools was not performed at regular intervals down the peat profile, a complete S budget for the peat monoliths could not be calculated. Some interesting insights were nevertheless gained from the data that were produced. In addition to the SO_4^{2-} pool being depleted in treatment monoliths (fig. 5.8), the elemental S (S^o) and acid-volatile S pools were also depleted while other, more recalcitrant S pools were enriched (although differences were not significant) (Table 5.2). This may indicate that relatively high rates of SO_4^{2-} reduction accelerate S turnover within inorganic S pools, which depletes labile S fractions while increasing more recalcitrant forms.

As well as investigating the forms of S in the peat, S residing in plant tissue was also examined (Table 5.3). It was found that aboveground vascular wetland plant matter might form a significant sink of the applied SO_4^{2-}, where the majority of S remains in the SO_4^{2-} form in vegetation from SO_4^{2-} treated monoliths. This sink may account for as much as 10% of the applied SO_4^{2-} and so plants may play a significant role in S cycling in acid rain impacted peatlands (Chapter 5)

7.6 Global implications of enhanced S deposition on CH_4 fluxes from wetlands

The work discussed so far provides strong evidence that SO_4^{2-} from acid rain is an important variable that reduces CH_4 emissions from peatlands impacted by commonly experienced rates of S deposition. The work provides insights into processes that influence the degree of suppression by acid rain on the CH_4 emission and advances hypotheses for the response of microbial communities to the pollution input. Here, the global implications of the findings presented are discussed (Chapter 6).

A regression model adaptation of a climate sensitive, global-scale model of CH_4 emission from natural wetlands (Walter, 1998; Walter and Heimann, 2000; Dlugokencky *et al*, 2001) was applied using two modelled climate forcing estimates from the NASA GISS GCM Ocean-Atmosphere Model (Russell *et al*, 2000) (one based on past and predicted changes in atmospheric greenhouse gases and the other including the relative cooling effect of atmospheric SO_4^{2-} aerosol) to estimate changes in the natural wetland source at decadal intervals between 1960 and 2080.

Global CH_4 emission data fields that were produced were combined with global fields of estimated total S deposition for the same years generated by a tropospheric S simulation in the NASA GISS GCM. Two simple interaction scenarios between S-

deposition and CH_4 emissions, based on the experimental findings presented in Chapters 3 and 5 were applied. The first (considered the less realistic of the two scenarios) assumes a step function in CH_4 flux suppression at 15 kg S $ha^{-1}yr^{-1}$. The second scenario assumed a linear suppressive effect of SO_4^{2-} at deposition rates lower than 15 kg-S ha^{-1} yr^{-1}, which is based on findings by Vile (*personal communication*) where SO_4^{2-} reduction rates in peatlands from a global S deposition gradient responded almost linearly within the range of 0-15 kg S $ha^{-1}yr^{-1}$.

The results show that the interaction between SO_4^{2-} deposition and CH_4 emission is potentially an important component of the contemporary global CH_4 budget. In northern wetlands, where experiments on which this exercise is based are located (>50°), modelled wetland CH_4 emissions are estimated to have been suppressed by 15 % of the regional source in 1980. Globally, it is estimated that between 22 and 28 Tg less CH_4 (depending on the climate forcing scenario) will be emitted from wetlands than would otherwise be emitted in a hypothetically 'S-pollution clean' world in 2030. This amount of CH_4 is similar in size to many other CH_4 budget components that have received far greater attention in the past. An implication of this is that since 1960, the effects of acid rain may have more than offset estimated growth in the natural wetland CH_4 source brought about through climate change (fig. 6.8). This offsetting effect is, however, estimated to be insufficient to prevent climate change induced increases in the global wetland source strength sometime between 2030 and 2080, as acid rain is predicted to decrease through pollution abatement legislation while the effects of climate change on CH_4 emissions, particularly in high latitudes (fig 6.6) becomes more pronounced. It should, however, be made clear that these estimates are only based on results of the few SO_4^{2-} deposition /CH_4 emission experiments that have been conducted thus far. In addition the interaction between SO_4^{2-} deposition and CH_4 emission at SO_4^{2-}

deposition rates of less than 15 kg-S ha^{-1} yr^{-1} is a major uncertainty that requires further investigation.

7.7 Recommendations for future work.

- The effects of enhanced SO_4^{2-} deposition through simulated acid rain has been shown to suppress CH_4 emissions from wetlands by around 40 % at, and above a deposition rate of 15 kg-S $ha^{-1}yr^{-1}$. Further long-term experiments, as reported in Chapter 3, with simulated SO_4^{2-} deposition rates of less than 15 kg-S $ha^{-1}yr^{-1}$ should be a priority if the role of pollutant S deposition on the global wetland source is to be fully understood.

- Similar long-term and low-dose SO_4^{2-} deposition simulation experiments, as reported in this thesis, should be conducted in wetlands spanning different climatic regimes. This will help to clarify the true response of wetland CH_4 emissions to changes in acid rain S-deposition.

- Processes that determine both the flux of CH_4 (methanogenesis and methanotrophy) and its suppression by acid rain SO_4^{2-} deposition stimulating an increased population of SRB should be investigated at regular time intervals, in both short-term and long-term experiments.

- Molecular microbiological techniques should be developed for quantitative investigations of methanogen and SRB community dynamics in low pH peatland systems. Such techniques may help in elucidating how microbial communities

in wetlands may respond to changes in S-deposition over the short and long-term.

- The findings presented in this thesis (Chapter 5) suggest that vascular plants may play an important part in seasonal S cycling in S impacted peatlands. This interaction requires further investigation.

- The extrapolation of the 'S-effect' on CH_4 fluxes, as reported in Chapter 6, is a first attempt at quantifying this interaction at the global scale. Improvements to the approach would be to include the effects of temperature and changes in water table on the interaction. Ultimately, alternate electron acceptors, such as SO_4^{2-}, and the processes operating within the S-impacted wetland soil/sediment column, should be characterised within the one-dimensional model from which global CH_4 emission is estimated (Walter and Heimann 2000). Global estimates would also be improved through more studies taking place in wetlands spanning a climatic gradient.

- The time scale over which peatland CH_4 emissions recover from a decrease in SO_4^{2-} deposition is an important uncertainty that deserves further investigation.

- Other pollutant species, such as NH_x and NO_y may also influence CH_4 emissions. They should, therefore, be examined for their potential effects on CH_4 emissions from wetlands, over the short and long-term with a similar approach to the studies reported in this thesis.

7.8 Summary and Conclusions

- Over two years of experimentally enhanced SO_4^{2-} deposition, at rates commonly experienced in acid rain-impacted areas, CH_4 emissions from peatlands were suppressed by around 40%.

- In the field experiment, there was no significant difference in suppression of CH_4 flux within the SO_4^{2-} deposition range of 25-100 kg-S $ha^{-1}yr^{-1}$. In a short-term controlled environment SO_4^{2-} manipulation experiment, the suppressive effect of SO_4^{2-} was found to be independent of the simulated deposition rate within range of 15-100 kg-S $ha^{-1}yr^{-1}$.

- Towards the end of the two-year field experiment at Moidach More, concentrations of dissolved CH_4 in peat pore water from the top 30 cm of the peat column were significantly smaller (by around 50%) in those plots treated with 50 kg-S $ha^{-1}yr^{-1}$ than in controls.

- The possibility that suppression of CH_4 fluxes may have been the result of a 'salt effect' was ruled out.

- After 18 months of SO_4^{2-} manipulation, sulfate reduction potential in plots treated with 50 kg-S $ha^{-1}yr^{-1}$ was found to be as much as 10 times larger than in control plots. This suggests that long-term suppression of CH_4 fluxes is the result of the formation of an enlarged population of competitively superior SRB.

- Temperature was found to control the extent of CH_4 flux suppression in acid rain impacted wetlands. Cooler temperatures stimulate an increase in the suppressive effect of SO_4^{2-} whereas during warmer periods, less of a SO_4^{2-} suppression was

observed. It is possible that temperature may effect competition between sulfate-reducing bacteria (SRB) and methanogens over mutual substrates.

- Water table was also found to control the extent of flux suppression with declining water table promoting a large suppressive response in CH_4 emissions. Two potential mechanisms may be responsible for this effect, either on their own or in combination. The first is that there is less dissolved CH_4 in SO_4^{2-} impacted pore water so that upon a lowering in water table, there is less CH_4 to come out of solution and so this contribution to the net emission will be smaller than in control plots. Secondly, there is proportionately more reduced S being re-oxidised to SO_4^{2-} in the enlarged aerobic zone of SO_4^{2-} -impacted peat than in controls. This may generate a pulse of SO_4^{2-} to fuel greater microbial competition in SO_4^{2-} treated plots.

- Very little of the applied SO_4^{2-} is re-emitted as volatile S compounds (<1%).

- Short-term suppression (over 2 months) of CH_4 emissions in response to a recent increase in applied SO_4^{2-} may reflect re-organisation of microbial community structure, possibly through a breakdown of methanogenic syntrophic associations between SRB and methanogens, before SRB populations have increased in number to form a competitively superior population.

- SO_4^{2-} concentrations were generally smaller in peat pore water from SO_4^{2-} -manipulated plots and monoliths than from controls. This is possibly the result of a stimulated SO_4^{2-} reducing community scavenging available SO_4^{2-}, thereby decreasing concentrations to below ambient levels.

- Globally, the effect of acid rain SO_4^{2-} deposition may be sufficient to reduce CH_4 emissions by as much as 22-28 Tg, a figure which places this interaction within the same size category as many other components of the global CH_4 budget that have received far greater attention.

- In northern peatlands (>50°) the effect of SO_4^{2-} deposition at 1990 rates may have been sufficient to reduce emissions from these systems by around 15% annually.

- The effects of acid rain on wetland CH_4 emissions during the second half of the 20th century may have been sufficient to offset climate change-induced growth in CH_4 wetland emissions during that time. Indeed the effect may have decreased the wetland CH_4 source to a level, which is below the estimated preindustrial source strength.

- The effects of future climate change on CH_4 emissions from wetlands are predicted to outweigh this offsetting effect of SO_4^{2-} sometime in the mid 21st century.

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Appendix

Mean CH4 fluxes from Moidach More – 1997-1998.

date	control	25kg	50kg	100kg
5/21/97	15.4	18.4	21.8	16.9
6/4/97	24.3	24.6	18.0	19.9
6/11/97	16.8	13.3	15.6	15.7
6/18/97	22.6	21.9	19.4	20.7
6/25/97	26.8	28.3	30.1	25.8
7/2/97	37.4	33.1	30.3	29.2
7/9/97	33.3	27.2	26.9	23.4
7/16/97	31.3	34.9	31.5	25.8
7/23/97	28.7	28.7	26.7	33.3
8/6/97	31.8	35.3	38.6	28.5
8/13/97	26.5	34.2	32.0	25.5
8/20/97	32.6	24.2	29.9	22.3
8/27/97	21.1	12.8	13.1	11.8
9/3/97	15.4	11.7	15.4	12.1
9/10/97	21.3	18.7	25.5	11.7
9/24/97	27.1	25.6	31.4	20.8
10/22/97	14.2	14.4	14.9	10.2
11/19/97	8.1	4.0	10.2	3.7
12/17/97	4.5	4.3	1.7	2.3
3/31/98	16.4	12.2	12.9	8.6
5/12/98	17.8	6.4	11.3	7.4
5/19/98	24.2	19.0	21.2	10.6
5/26/98	28.4	13.6	23.3	11.2
6/2/98	27.5	21.1	21.4	19.0
6/9/98	48.9	37.0	49.6	38.2
6/16/98	62.6	49.5	52.3	39.5
6/23/98	46.2	34.6	44.1	34.4
7/1/98	66.6	42.8	52.7	35.7
7/7/98	50.5	24.7	28.3	20.6
7/14/98	51.9	32.5	40.2	23.8
7/21/98	46.8	43.9	66.4	54.6
7/28/98	100.6	63.1	89.5	78.4
8/4/98	23.3	33.9	29.3	29.0
8/11/98	110.5	129.5	146.2	104.0
8/18/98	48.1	27.8	33.5	36.9
8/25/98	133.0	103.1	143.3	89.8
9/8/98	66.9	67.0	55.3	60.1
9/15/98	89.9	74.2	115.9	56.7
9/22/98	132.0	104.9	106.5	90.8
9/29/98	139.7	97.0	98.3	94.4
10/7/98	124.9	86.5	99.6	81.1
10/13/98	103.5	78.8	87.4	71.4
10/20/98	31.2	11.1	18.0	25.9

10/28/98	39.8	32.0	41.6	24.5
11/11/98	35.8	22.4	40.2	28.2
12/2/98	40.9	27.8	42.6	31.6